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Life History and Smolt-to-Adult Survival of Keogh River Steelhead Trout (*Salmo gairdneri*) and the Relationship to Smolt Size

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Ward, B. R., and P. A. Slaney. 1988. Life history and smolt-to-adult survival of Keogh River steelhead trout (*Salmo gairdneri*) and the relationship to smolt size. Can. J. Fish. Aquat. Sci. 45: 1110–1122.

A decade of data on wild steelhead trout (*Salmo gairdneri*) from a coastal stream in British Columbia demonstrated large fluctuations in smolt number, age structure, size, estimates of adult run sizes, smolt-to-adult survival, and adult age. Adult runs averaged 922 (range 209–2730) with approximately 10% repeat spawning incidence. Females repeat spawned more than males and were more abundant as kelts, but maiden run adults were equally male and female. The proportion of males returning after 1, 2, and 3 yr in the ocean averaged 3, 62, and 35%, respectively; 58 and 42% of females returned after 2 and 3 yr, respectively. Adult age structure, smolt number, and smolt size varied biennially. Adult size decreased with freshwater age, but increased with ocean age of returns. Males were larger at each ocean age. Mean number of smolts (50:50 sex ratio) was 5543 and varied fivefold. Mean smolt length was 173 mm and mean weight was 49 g. Smolts were 2–5 yr old, and freshwater age 3 was most prevalent (average 56%). Mean survival from smolt to adult was 16% (7% from 1978 cohorts to 26% from 1982 cohorts). Survival was positively correlated with smolt length and weight. However, 1982 cohorts had twice the survival of other cohorts, possibly related to El Niño. No clear relationship was found between return age and mean smolt size, but on average, freshwater age was inversely related to ocean age. The relationships suggest that predictive models may be developed over the longer term from this type of study.

L'analyse des données recueillies pendant une décennie sur la truite arc-en-ciel sauvage (*Salmo gairdneri*) peuplant un cours d'eau côtier de la Colombie-Britannique a révélé d'importantes fluctuations du nombre, de la structure des âges et de la taille des smolts, et a donné des estimations du nombre de géniteurs amontants, de la survie des smolts jusqu'à l'état adulte et de l'âge des adultes. Le nombre moyen de géniteurs amontants s'élevait à 922 individus (écart : 209–2730) dont environ 10 % étaient des géniteurs multifrai. Les femelles ont frayé une seconde fois plus souvent que les mâles et étaient plus abondantes à l'état de charognard; toutefois, les remontes d'individus vierges étaient constituées d'autant de femelles que de mâles. Le nombre relatif de mâles qui sont revenus en eau douce après avoir passé 1, 2 et 3 ans en mer s'élevait en moyenne à 3, 62 et 35 % respectivement; 58 et 42 % des femelles sont revenues après avoir passé 2 et 3 ans respectivement en mer. La structure des âges des adultes et le nombre et la taille des smolts ont varié tous les deux ans. La taille des adultes a diminué en fonction de la période passée en eau douce mais a augmenté en fonction de l'âge des individus amontants. Pour chaque âge océanique, les mâles ont montré une plus grande taille. Le nombre moyen de smolts (proportion relative des sexes 50:50) s'élevait à 5 543 et variait de 2 104 à 11 897 individus. La longueur et le poids moyens se situaient respectivement à 173 mm et 49 g tandis que l'âge allait de deux à cinq ans. Les individus qui avaient passé trois ans en eau douce étaient les plus abondants (moyenne 56 %). Le taux de survie moyen du stade smolt au stade adulte s'élevait à 16 % (7 % chez les cohortes de 1978 et 26 % chez les cohortes de 1982). Le taux de survie était en corrélation positive avec la longueur et le poids des smolts. Toutefois, les cohortes de 1982 ont montré des taux de survie deux fois supérieurs à ceux d'autres cohortes, peut-être en réaction au courant El Niño. Aucune relation précise n'a été relevée entre l'âge au retour et la taille moyenne des smolts mais, en moyenne, l'âge en eau douce était en relation inverse avec l'âge en mer. Les relations signalées portent à croire que des modèles prédictifs pourront être élaborés à long terme à partir de résultats de telles études.

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Information on marine survival rates of wild Pacific salmonids, particularly wild steelhead trout (*Salmo gairdneri*), is sparse. Also, life history studies have been mainly based on scale samples from anglers' catches (Maher and Larkin 1954; Withler 1966; Narver 1969; Narver and Withler 1971). Earlier attempts at enumerations of wild steelhead suffered from a lack of discrimination of parr from smolt migrants (e.g. Shapavalov and Taft 1954) or from low numbers of samples and size-selective trapping (e.g. Peterson and Lyons 1968). Current studies in northwest Washington have produced more promising results on smolt-to-adult survival from small streams (T. H. Johnson and R. Cooper, Washington Department of Game, Port Townsend, WA, pers. comm.).

Time series data from salmonid migration studies are a useful tool in understanding and managing wild populations. The 10-yr study at Waddell Creek, California (Shapavolov and Taft 1954), provided information on juvenile and adult timing and run size, fish age and size, sex ratio of adults, and factors influencing migrations. Similar results were not available for a British Columbia steelhead population, and since productivity of

fish and life history components likely differ, there was a need to examine British Columbia's smolt and adult migrants more closely.

Similar to Atlantic salmon (*Salmo salar*), steelhead life history is complex with variable ocean age and return rates of adults, making it difficult to predict run size (Chadwick 1982). Given an expected return of adults, it is difficult to estimate how many return as ocean age 1, 2, or 3 and whether males return differently from females. Previous studies had not attempted to explain the relative returns of different steelhead age groups. There was a need to more closely define the influence of smolt size on marine survival and age at return. Walters et al. (1978) noted a need for better definition of the size and survival relationship in salmon of the Pacific coast, and Hyatt and Stockner (1985) commented on the importance of this type of examination for management purposes.

The purpose of this study was to examine in steelhead trout the variability in smolt and adult numbers, size, and age structure at a coastal stream in British Columbia; to examine the relationship between smolt number and adult returns; and to

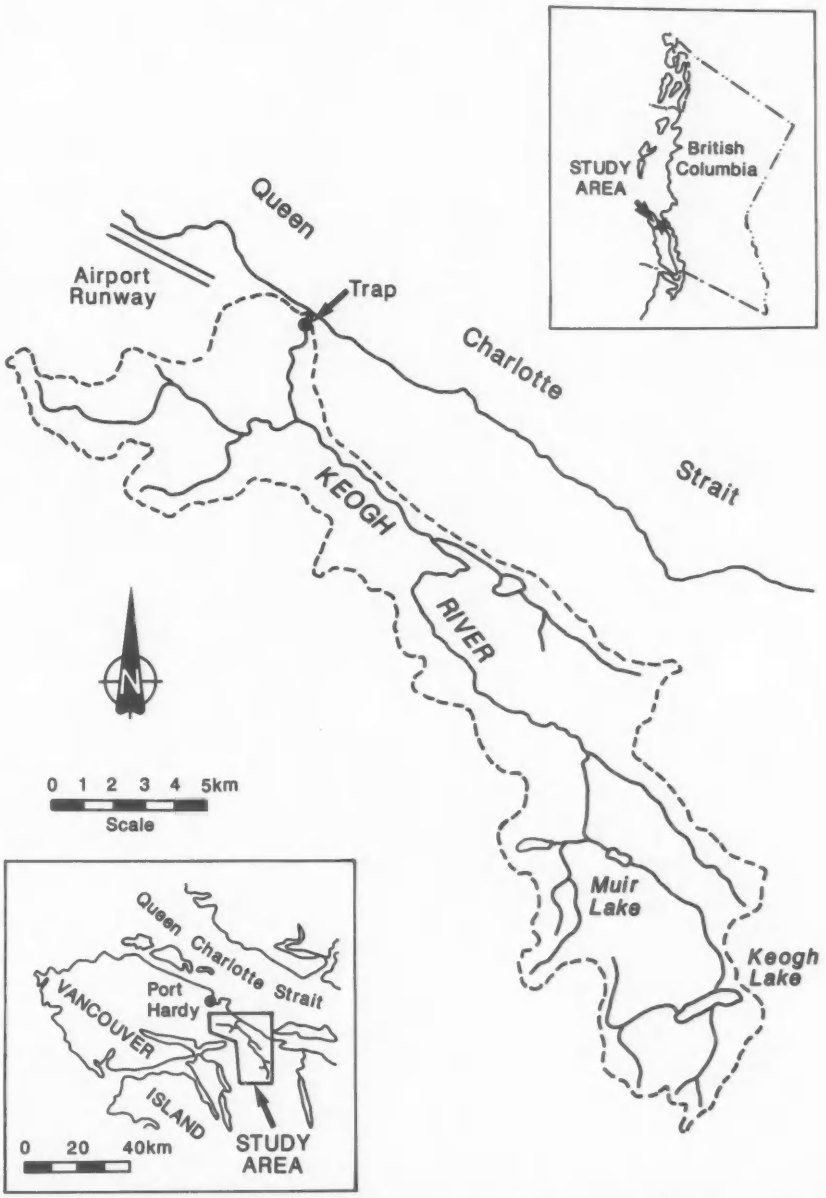

FIG. 1. Keogh River drainage area showing general location on the British Columbia coast and northern Vancouver Island.

determine the effect of smolt size on survival rate and ocean age at maturity. These relationships were combined in a predictive equation to examine their overall role in describing run sizes of adult steelhead of the Keogh River, with the longer term goal of developing predictive models based mainly on smolt yield to estimate adult returns.

Methods

Study Area

The Keogh River, near Port Hardy on Vancouver Island, British Columbia, flows northeast for 33 km and drains into Queen Charlotte Strait (127.4° W, 50.6° N; Fig. 1). Mean annual discharge is 5.3 $m^3 \cdot s^{-1}$ and ranges from 0.1 to approximately 254 $m^3 \cdot s^{-1}$ from the 129-km^2 watershed. Flow is lowest in summer and peaks in late autumn and winter, the regime mainly controlled by rainfall (mean annual precipitation 178 cm, Environment Canada). Dissolved solids and nutrients are low: total dissolved solids, 30 $mg \cdot L^{-1}$; orthophosphorus, 0.001 $mg \cdot L^{-1}$; nitrate-N, 0.01 $mg \cdot L^{-1}$. Mean annual stream temperature is 9°C and ranges from near 0 to 21°C (Ward and Slaney 1979, 1981).

Forest cover is typical of the region, dominated by western red cedar (*Thuja plicata*) and western hemlock (*Tsuga heterophylla*). Approximately 35% of the drainage area has been forest harvested (clearcut) in the past 30 yr (see Perrin and Johnston (1985) for more detailed description of the watershed).

Streams or lakes in the watershed support populations of pink salmon (*Oncorhynchus gorbuscha*), coho salmon (*O. kisutch*), chum salmon (*O. keta*), steelhead trout, anadromous and resident Dolly Varden char (*Salvelinus malma*), resident and anadromous cutthroat trout (*Salmo clarki clarki*), kokanee (*O. nerka*), coast range sculpin (*Cottus aleuticus*), prickly sculpin (*C. asper*), threespine stickleback (*Gasterosteus aculeatus*), and Pacific lamprey (*Lampetra tridentatus*).

Angling pressure is low and is focused on steelhead during autumn to early spring (about 500 angler days), coho during autumn, Dolly Varden during midsummer to early spring, and periodic summer fishing for cutthroat trout in lakes and pink salmon at the river mouth in the late summer. Prior to implementation of catch and release regulations (1980) on Vancouver Island, annual steelhead harvest was approximately 5% at the more remote Keogh River, based on creel surveys (data on file). A commercial fishery in the ocean directed at salmon intercepts some steelhead kelts in late spring (Evans 1979). Mortality as a result of sports fisheries was assumed to have insignificant effects on the results presented, or affected marked and unmarked fish equally (i.e. anglers caught and removed marked and unmarked fish with equal chance).

Marked hatchery smolts (range 3100–30 400) were introduced to the Keogh system beginning in 1979, released mainly near the mouth (Slaney and Harrower 1981), and may have resulted in slightly altered smolt-to-adult survival of wild fish. Whole-river nutrient enrichment experiments (Perrin et al. 1987) began May 15, 1983, but effects to the 1983 and 1984 smolt migrations were not detectable.

Adult Sampling

Adult steelhead migrating upstream were trapped from 1975 to 1986 at a counting fence 300 m from the river mouth (Mottram 1977; Johnston et al. 1986). Fish were usually captured from February to May except 1976–77 when the complete season of adult upstream migration was sampled from November to May. The total number of adult males and females was estimated by mark and recapture using the adjusted Petersen estimate (Ricker 1975) by marking upstream migrant adults and capturing kelts during their downstream migration through the trap from March to June. Adults were sexed by external features and marked by either an adipose fin clip or by punching a round hole (6 mm) in the operculum. Fork lengths were measured and scale samples removed, similar to Maher and Larkin (1954). Because of differential mortality of adult males and females from the time of river entry to kelt exit, male and female numbers were estimated separately.

The second year of trap operation (November 1976 to June 1977) enabled comparison of early-run (November–January) to late-run (February–May) survival of adults to kelt by marking adults with an adipose fin clip and inserting numbered Floy tags in front of the dorsal fin. The Petersen estimate assumes an equal chance of catching all fish. A bias to early- or late-run composition in the kelts, because of differential mortality, could affect the population estimates. The distribution of marked fish in the kelt catch was examined to detect this possible bias.

Scales were prepared for age determination by making plastic impressions (Narver and Anderson 1974) and then projected onto a 3M500 Microfiche reader–printer (model 275 AGM) to photomicrograph. Ocean age of wild adults was validated by comparison of scale patterns with marked hatchery returns beginning in 1982. Two readers independently read scales, conferred, and mediated on differences. The European system of age designation was utilized, where 2.3 represents a fish of river age 2 and ocean age 3. Age composition of adults was examined for each sex for both freshwater and ocean years and repeat spawning frequency. Size differences between sexes were compared for each age class. The association between freshwater and saltwater age for males and females during 1976–85 was examined.

Smolt Sampling

Steelhead smolts migrating downstream were enumerated from early April to mid-June, 1977–86. Trapping during the autumn and winter of 1975 and 1976 indicated that there were no autumn/winter smolts. Migrants were collected in trap boxes after passage over a large area of horizontal screen (Slaney 1977) or in freshets by additional operation of hanging inclined plane (i.e. Wolfe) traps (Mottram 1977). Total numbers were estimated by conducting a series of trap efficiency tests with large (> 12 cm) coho smolts marked and released upstream, similar to deHrussoczy-Wirth (1979). Trapping efficiency was typically 90% (range 70–100%). Steelhead smolts were not utilized to test trap efficiency because sampling with seines upstream of the trap during June–July (several weeks after completion of the smolt migration) indicated that a proportion of a marked (n = 200) test group reverted to parr (n = 5), failing to migrate. Although treated the same, marked steelhead smolts were recaptured at a rate 17% lower than marked coho smolts (see Hoar 1976). Since run timing and size of coho smolts differed from steelhead smolts, the trap efficiency tests served as a check on trap operation and an approximation of our ability to capture steelhead smolts. The fence was inspected daily to prevent smolt passage anywhere but through the trap boxes.

Smolts were randomly sampled for fork length and weight and subsampled for scales (1977–79). After 1980, smolts were stratified random sampled for scales by 10-mm size intervals

TABLE 1. Population estimates of Keogh River steelhead runs.

Year		Marks	Captures	Recaptures	Others[a]	Estimate	−95% CL[b]	+95% CL	% of *N* handled
1976	Males	98	97	39	2	242	179	337	65
	Females	187	196	59	3	617	481	791	53
	Total					859	709	1057	
1977	Males	180	39	24	3	291	200	442	68
	Females	246	91	68	6	334	266	420	82
	Total					625	511	799	
1978	Males	81	39	8	7	367	200	743	32
	Females	132	125	50	13	339	262	451	65
	Total					706	522	1098	
1979	Males	79	44	29	20	138	103	194	82
	Females	111	82	68	27	161	133	196	95
	Total					299	254	365	
1980	Males	53	33	21	9	91	64	138	81
	Females	70	56	41	23	118	93	154	92
	Total					209	172	268	
1981	Males	109	92	30	13	340	244	491	54
	Females	109	132	60	30	268	215	334	79
	Total					608	498	773	
1982	Males	130	94	24	9	503	346	746	42
	Females	195	180	88	38	435	361	525	75
	Total					938	764	1197	
1983	Males	150	99	8	20	1687	913	3429	15
	Females	187	239	44	46	1043	794	1406	41
	Total					2730	1917	4509	
1984	Males	175	163	42		667	497	917	44
	Females	249	360	132	1	677	572	801	71
	Total					1344	1144	1623	
1985	Males	100	45	15	9	296	187	498	47
	Females	133	84	41	14	283	214	386	67
	Total					580	451	807	
1986	Males	169	137	39	8	591	405	917	47
	Females	220	215	74	12	646	520	808	58
	Total					1237	1014	1603	

[a]Trap mortalities, hatchery broodstock, or escapees.
[b]Ricker (1975) Appendix II.

(Ricker 1975). Techniques for preparation and reading of smolt scales for age were similar to those for adult scales. Previous ageing of scales collected by electrofishing and seining parr throughout the river assisted recognition of annuli in smolt scales. Smolt sex ratios were examined in 1980, 1984, and 1985 by sacrificing subsampled smolts and examining the gonads. Mean weights were calculated by converting lengths to weights based on length–weight regressions (n = 88–615) established annually.

Smolt-to-Adult Survival

Marine survival rates were calculated as the sum of male and female return estimates from a given smolt year divided by that year's number of migrant smolts. Returns were estimated after calculating the ocean age composition derived from adult scales and the estimated adult population size. The returns of smolt cohorts were the sum of the ocean age 1 returns (jacks), the ocean age 2 returns in the next year, and the ocean age 3 returns in the following year. The repeat spawning component was removed from the adult population estimate; survival rates were only based on maiden fish (i.e. first time spawners). The method of calculating marine survival by smolt year differs from that of Hyatt and Stockner (1985); they calculated sockeye marine survival based on the brood year of origin. Sockeye smolts are composed almost entirely of 1 age class whereas steelhead smolts are several ages.

The effect of smolt size on survival rate in the ocean was examined by least squares regression analysis using the mean smolt length and weight in a smolt cohort. Seven years of smolt enumeration and sampling (1977–83) and 9 yr of adult return estimation and aging (1978–86) were available.

Marine survivals were compared between years by adjusting the mean smolt length to a standard. The regression of mean

smolt size on marine survival was used as a statistical control (Sokal and Rohlf 1981). This procedure provided an indication of the variability in ocean conditions as reflected by the adjusted survival rates, assuming that differences in size-adjusted survival were mainly a result of conditions in the ocean.

Results

Adult Steelhead

Poplation sizes

The mean estimate of wild steelhead (winter run) returning to the Keogh River from 1976 to 1986 was 922 and ranged between 209 and 2730 (Table 1). The recapture rate of marked adults as kelts was different for each sex. The mean rate of recapture of male kelts was 23% of the fish marked as upstream migrants (range 5–40%) whereas mean recapture rates of females was about two times higher or 42% (range 24–61%). Accordingly, different mortality rates of males and females justified stratifying population estimates by sex. The recapture rate for males was poor in 1983 (5.3% of the fish marked) and confidence limits were wide on this estimate (913–3429). Confidence intervals for males were an average 31% greater than for females. The number of adults captured and marked moving upstream was usually slightly higher than the number of kelts captured migrating downstream and examined for marks. On average, 62% (SD = 20.3; Table 1) of the estimated population was handled either as upstream migrant spawners, unmarked downstream migrant kelts, broodstock, or trap mortalities.

Bias to late-run fish in marking coinciding with higher postspawning mortality of early-run fish would have lowered the population estimates. Comparison of the recapture rate of early-run versus late-run fish, which were adipose-clipped and given numbered Floy tags, indicated only a slightly higher (2.3%) postspawning survival of the late-run group in the 1976–77 season.

Repeat spawning

Repeat spawning frequency averaged 8.1 and 11.6% for males and females, respectively, within 1976–86 return years (Table 2) and rates were similar to the above when expressed as a percentage of the previous year's run. Repeat spawning was higher for females (up to 31%) in the overall pooled estimate ($p < 0.05$). Male repeat spawners outnumbered females in only 2 of 11 years (1983 and 1985). There was no correlation between the kelt catch (spawned fish that emigrated) and the next year's repeat spawning males ($r^2 = 0.15$, $p > 0.05$) and females ($r^2 = 0.03$, $p > 0.05$). Condition of kelts (e.g. scars, fungal growth) upon exit varied between years (data on file) and may have influenced subsequent survival.

Adult sex ratios

On average, the percentage of males and females was similar ($p > 0.05$): 48% males (SD = 9.3) and 52% females (SD = 8.5). However, there was significant departure from a uniform sex ratio in adult estimates in three years ($p < 0.05$). In 1976 and 1985, females outnumbered males by 38 and 33%, respectively, whereas in 1983 there were 23% more males.

TABLE 2. Age structure of adult steelhead populations immigrating the Keogh River during return years 1976–86.

	% Repeat spawners	Maiden fish	Age composition (%)				No./age			
			.1	.2	.3	.4	.1	.2	.3	.4
Males										
1976	20.6	192	2.6	49.4	48.1		5	95	92	
1977	2.9	283	0.7	82.2	17.0		2	232	48	
1978	11.1	326	3.1	45.9	50.0		10	150	163	
1979	6.0	130	1.1	87.2	11.7		1	113	15	
1980	3.5	88	3.6	45.5	50.9		3	40	45	
1981	2.4	332	0.0	87.8	12.2		0	291	40	
1982	8.9	458	14.6	28.2	57.3		67	129	263	
1983	3.3	1631	4.2	90.9	4.9		69	1483	80	
1984	7.3	618	1.4	55.6	43.0		9	344	266	
1985	15.6	250	1.6	50.0	48.4		4	125	121	
1986	7.7	549	0.0	60.4	39.6		0	332	217	
Mean	8.1	442	3.0	62.2	34.8		15	303	123	
Females										
1976	31.0	426		38.6	60.7	0.7	0	164	258	3
1977	10.4	299		89.4	10.6		0	267	32	0
1978	17.5	280		36.4	63.6		0	102	178	0
1979	6.2	151		83.6	14.8	1.6	0	126	22	2
1980	5.6	111		42.9	56.0	1.2	0	48	62	1
1981	5.1	254		89.4	10.6		0	227	27	0
1982	12.1	382	0.6	29.7	69.7		2	113	266	0
1983	2.6	1016		86.3	13.7		0	877	139	0
1984	11.4	600		46.2	53.8		0	277	323	0
1985	14.5	496		53.0	47.0		0	263	233	0
1986	11.1	576		44.0	56.0		0	253	323	0
Mean	11.6	402	0.1	58.1	41.5	0.3		247	169	1

TABLE 3. Ocean age composition of male and female steelhead adults returning from 1976 to 1985 expressed as percentage within freshwater age groups.

	Freshwater Age	Ocean age .1	.2	.3	.4	*n*
Females	2.		52.6	46.6	0.8	249
	3.		65.7	34.1	0.2	501
	4.		79.6	20.4		93
Males	2.	0.6	63.7	35.7		168
	3.	3.0	69.6	27.4		336
	4.	13.6	68.2	18.2		44
						1391

Age in ocean years

In general, males returned at an earlier ocean age than females. Although jacks were present (mean 3%), females age .1 were very rare (Table 2). Composition of males aged .2 was 4% higher on average than .2 females, but females were predominant by 7% in composition of .3. Age .4 were present in insignificant numbers (average 0.3%, females).

There was a biennal pattern whereby age .2 were mainly dominant in odd-numbered years and .3 were dominant in even-numbered years ($p < 0.05$). Females of age .3 displayed the greatest difference between odd- (average composition 19%, SD = 15.6) and even-numbered (average composition 60%, SD = 6.0) years. However, the returns since 1985 did not exhibit this pattern and .2 and .3 groups were similar in percent occurrence (Table 2).

Age in freshwater years

Freshwater age composition from adult scales was difficult to sample as a result of regenerated scales in the freshwater growth zone (approximately 30%). However, by combining adults returning from 1976 to 1985, several differences were apparent in the combined freshwater and saltwater age composition. Male and female adults were predominantly aged freshwater 3's at 61 and 59%, respectively. As freshwater age increased, the proportion of total adults returning after 3 yr in the ocean decreased, particularly for females (this is discussed further in the section on age at return of cohorts). Age .1 and .2 males and .2 females increased in percent occurrence as freshwater age increased (Table 3). No adults returned with freshwater age 0 (fry) or 1 scales ($n = 1393$) and freshwater age 5 was very rare (one male and one female, both returning as .2), as was age .4 ($n = 3$).

Adult length

Differences in adult mean length appeared to be associated with the year of smolting, age, and sex. Adult males were 28–48 mm larger within ocean ages .2 and .3 than adult females ($p < 0.05$; Table 4). Both male and female lengths were different ($p < 0.05$) between years. Fish were grouped by year of smolting (1977–83), and using the GT-2 method (Sokal and Rohlf 1981), the order of mean lengths was tested. The pattern for age .2 males and females was similar in that adults derived from the 1977, 1980, and 1981 smolt cohorts were significantly larger than the adults derived from other smolt years. Size of adults decreased ($p < 0.05$) as freshwater age increased (Table 4). Closer examination showed that within the .2 adults, length slightly increased ($p < 0.05$) with freshwater age, yet for .3 adults, length was similar in the freshwater age 2 and 3 groups but lower from adults (by 12 mm for females and 42 mm for males) that had spent 4 yr in freshwater ($p < 0.05$; Table 4).

TABLE 4. I. Adult size at ocean age. Mean length ranked low to high with nonsignificantly different means underscored by the same line.

Females aged .2							
Smolt year	1978	1982	1979	1983	1981	1980	1977
$\bar{x}$ length	650	654	663	663	679	680	690
Males aged .2							
Smolt year	1983	1982	1979	1978	1980	1981	1977
$\bar{x}$ length	680	685	686	688	699	703	715
Females aged .3							
Smolt year	1983	1982	1978	1979	1981	1977	1980
$\bar{x}$ length	753	770	775	777	778	782	792
Males aged .3							
Smolt year	1978	1980	1983	1979	1982	1981	1977
$\bar{x}$ length	754	777	805	814	824	833	847

TABLE 4. II. Adult size at freshwater age and ocean age of steelhead (SD = standard deviation, *n* = sample size) for all upstream migrants sampled from 1977 to 1983 in the Keogh River, B.C.

Freshwater age	Sex		Ocean age .1	.2	.3	All
2	F	Length	—	661	775	716
		SD	—	36	43	70
		n	—	130	116	248
	M	Length	430	693	837	742
		SD	—	45	58	88
		n	1	107	59	167
3	F	Length	—	676	778	711
		SD	—	36	41	62
		n	—	326	171	498
	M	Length	481	700	835	730
		SD	34	38	66	88
		n	10	233	91	334
4	F	Length	—	682	766	699
		SD	—	37	35	50
		n	—	73	19	92
	M	Length	450	708	793	688
		SD	74	30	46	109
		n	6	30	8	44
All	F	Length	470	673	777	714
		SD	—	38	41	64
		n	1	823	518	1346
	M	Length	476	701	825	731
		SD	50	41	63	89
		n	32	610	281	924

Smolts

Population sizes

The mean number of wild smolt migrants was 5543 from 1976 to 1983 (Table 5). Variation between years was substantial in both numbers (2104–11 897) and age class structure (2–5 yr in freshwater; Table 5). Even years produced fewer smolts than the preceding odd year ($p > 0.05$), and this was most pronounced in age 3 and 4 smolts. On average, fish smolted after 3 yr in freshwater (56% of composition) at the Keogh River.

TABLE 5. Steelhead smolt yield of the Keogh River (1977–83) and estimated number/freshwater age from percent composition in scale analysis.

Year	Yield	Trap efficiency	Freshwater age 2 (%)	3 (%)	4 (%)	5 (%)	Scale n^a
1977	2 344	70	909 (39)	1207 (52)	227 (10)	0 (0)	103
1978	2 104	78	1117 (53)	789 (38)	198 (9)	0 (0)	32
1979	6 900	94	835 (12)	4892 (71)	1173 (17)	0 (0)	141
1980	5 500	90	1546 (28)	3350 (61)	561 (10)	44 (1)	128
1981	11 897	90	3474 (29)	5592 (47)	2713 (23)	118 (1)	514
1982	4 000	90	1504 (38)	2376 (59)	104 (3)	16 (0)	205
1983	6 053	90	1951 (32)	3701 (61)	401 (7)	0 (0)	177
Mean	5 543		1619 (33)	3130 (56)	768 (11)	25 (0)	

[a]Readable, nonregenerated scale samples

Smolt size

Mean annual size of steelhead smolts was 173 mm and 49 g with a range between 160 and 187 mm and 40 and 67 g. Size displayed odd and even cyclic dominance similar to smolt number and ocean age composition, larger mean smolt size occurring in odd-numbered years ($p < 0.05$; Fig. 2). Smolt size was directly related to smolt age (Tables 5 and 6). Length–weight regressions and condition factors were not significantly different between years ($p > 0.05$). There was little annual variation in mean smolt length within freshwater ages. However, in 1980, age 3 and 4 fish were much smaller ($p < 0.05$) than other years (Table 6) and there was a trend to larger sized age 3 smolts in odd-numbered years ($p < 0.05$, Mann–Whitney *U*-test), but this did not apply to the other ages ($p > 0.05$). For all years, mean smolt length at freshwater age was 153, 177, and 218 mm for age 2, 3, and 4 smolts, respectively. These means were derived from a truncated normal distribution (no fish less than 130 mm) which was skewed to the right. Corresponding mean weights at these average lengths were as follows: age 2, 33 g; age 3, 48 g; age 4, 83 g. High variability in age class strength was evident.

The weighted mean length calculated from smolt number and size at age was the same as the mean smolt length from length frequency distributions in all but two years: in 1978 and 1979 the weighted means from scales were 4 and 2% larger, respectively, than means from smolt length frequency. Some smolt length intervals were unavailable in the scale sampling of 1978 because of a small sample size, and a slight bias to more intensive sampling of larger smolts may have affected the 1979 results.

Sex of smolts

In 3 yr of smolt samples, the percent composition of males was lower than females by about 4% (Table 7). However, sex of smolts was not statistically different from 1:1 ($p > 0.05$, *G*-test). With all years lumped and compared by size interval, a trend to more females in the smaller categories and more males in the largest interval was suggested, but a doubling of sample size would be required for this difference to achieve statistical significance. Similarly, sacrificed smolts from 1985 were equally male and female within freshwater ages 2, 3, and 4.

Survival in the ocean

Marine survival estimates, based on maiden-run fish only (i.e. repeat spawners excluded), from smolt year classes 1977–83 ranged from 7 to 26% (Table 8). Variation in adult return was a linear function of smolt numbers emigrating from the river (Fig. 3) from 1977 to 1983 ($r^2 = 0.86$, $n = 7$, $p < 0.05$). This relationship was highly dependent on the 1981 smolt data point; several more years of data would be required to describe this relationship with confidence. The mean survival was 16.2%

Variation in the smolt-to-adult survival could be accounted for by differences in mean smolt length or weights between years (Fig. 4). Data for 1977–83, excluding 1982 as an outlier, suggested that the relationship was linear for the range of mean smolt lengths and weights such that

(1) Survival = 5.75 (length (cm)) − 84.95 ($r^2 = 0.95$, $p < 0.05$)

or

(2) Survival = 0.60 (weight (g)) − 15.35 ($r^2 = 0.95$, $p < 0.05$).

The coefficient of determination was much reduced when the 1982 smolts were included in the regression analysis (length, $r^2 = 0.47$, $p > 0.05$; weight, $r^2 = 0.36$, $p > 0.05$). The absolute value of the residual of the 1982 datum in the regression was more than twice the value of the next closest absolute value of the residuals and thus can be considered an outlier (Draper and Smith 1966). Size-adjusted comparisons indicated very little difference between years in marine survival except the 1982 smolt year class which was 104% greater than the expected survival. Comparing smolt sizes in 1978 and 1982, the difference in survival was expected to be about 6%; instead, a difference of 19% was recorded. When other smolt survival rates were statistically size-adjusted this way, differences in survival were at most 3%, indicating the aberrant nature of the 1982 cohorts' marine survival.

Cohort age at return

No clear pattern was discernible in the sea age at return of male or female smolt cohorts (Table 8). Variation in age composition in ocean years was broad. Males aged .1 were relatively more abundant from 1982 smolts, and conversely, males aged .3 ($\bar{x} = 37\%$) were lowest from 1981 and 1982 smolts. Although some males were age .1, no females were, and age composition of .2 and .3 males and females was similar ($p > 0.05$). Females aged .3 were also lowest from 1981 smolts (Table 8), but slightly higher than average ($\bar{x} = 46\%$) from 1982 smolts. Estimates of age composition of males and females appeared to be correlated but points were scattered ($r^2 = 0.64$, $p > 0.05$).

There were no significant relationships of mean smolt size and proportion returning at age .3 for males (length, $r^2 = 0.09$,

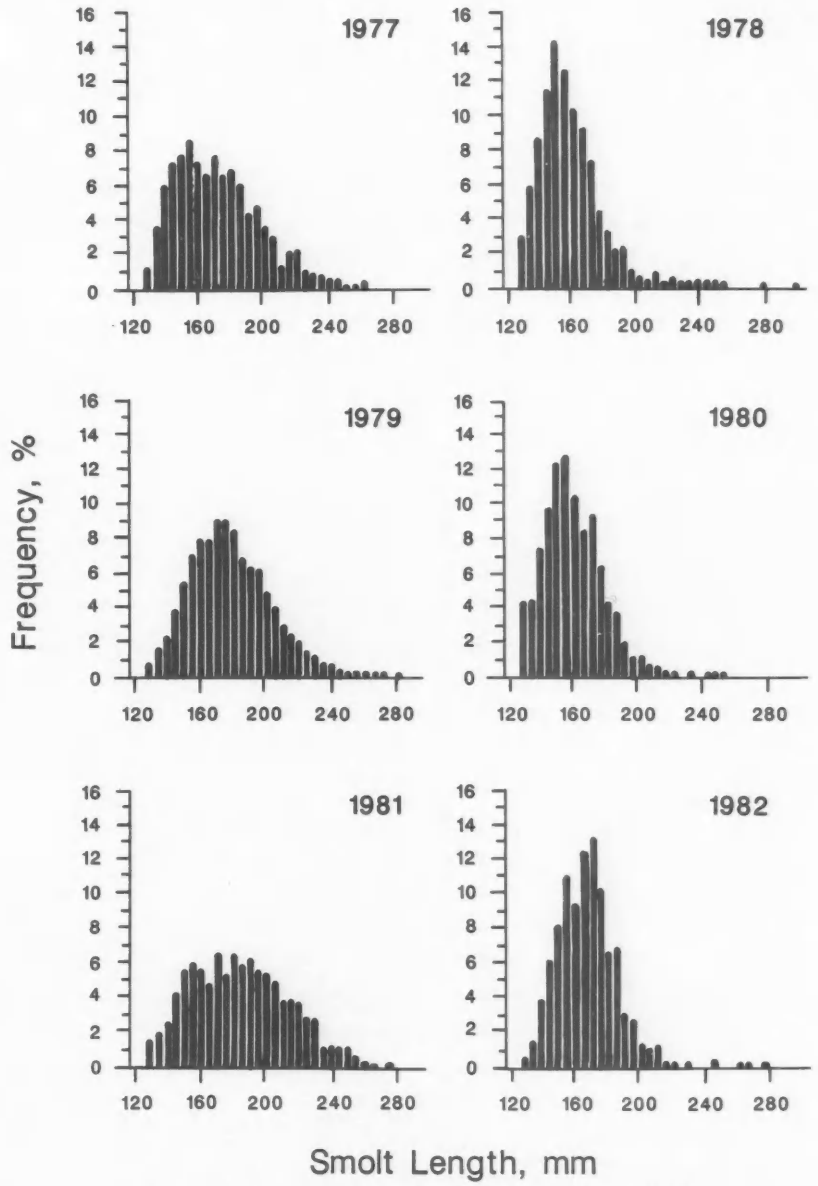

FIG. 2. Length frequency distributions of steelhead smolts, 1977–82.

$p > 0.05$; weight, $r^2 = 0.2$, $p > 0.05$) or females (length, $r^2 = 0.07$; weight, $r^2 = 0.2$, $p > 0.4$). However, when estimates of marine survival were high, males of age .3 appeared to decline in relative abundance ($r^2 = 0.42$, $p > 0.05$)). Females did not show this trend ($r^2 = 0.1$, $p > 0.5$) and neither slope was significant ($p > 0.05$). The influences of freshwater age and growth may confound this analysis.

The age at return was not influenced by size or growth in males and females in the same manner. If adult size is a determining factor in the age at return, then age should vary with changes in marine conditions that affect growth. Length at ocean age was significantly different in many cohorts (Table 4), part of which may be accounted for by the differences in smolt length or age. When mean smolt length was subtracted from the size at ocean age, only males indicated an inverse relationship of growth in the ocean and age at return (Fig. 5). Females did not vary as much in length nor did they mature earlier with increased growth, compared with males. Again, freshwater age effects may confound this analysis.

There was a relationship of freshwater age with ocean age (Table 3). Since age at return decreased with increased smolt age, we assumed that age at return also decreased with increased smolt size. If the relationship between percentage returning at age .3 and size at freshwater age is linear, then the relationship can be described by the line

TABLE 6. Steelhead smolt size upon emigration from the Keogh River.

Year	Mean length (mm) (SD)	Length sample size	Mean weight (g) (SD)	Length (mm) at freshwater age 2 (SD)	3 (SD)	4 (SD)	5 (SD)
1977	174 (26)	1601	48 (26)	152 (16)	180 (21)	221 (23)	—
1978	160 (21)	1684	40 (18)	149 (12)	176 (23)	221 (12)	—
1979	180 (24)	6246	56 (25)	151 (12)	179 (19)	225 (31)	—
1980	161 (17)	1337	41 (25)	151 (14)	164 (13)	191 (19)	252 (—)
1981	187 (30)	3037	67 (23)	156 (14)	186 (16)	226 (18)	281 (9)
1982	170 (18)	840	44 (20)	153 (8)	177 (11)	222 (22)	272 (9)
1983	176 (21)	967	47 (18)	160 (13)	180 (16)	218 (17)	—
Average	173		49	153	177	218	268

(3) $P_m = 82.04 - 0.303\,(L)$ ($r^2 = 0.99$, $p < 0.05$)

for males and

(4) $P_f = 105.88 - 0.395\,(L)$ ($r^2 = 0.99$, $p < 0.05$)

TABLE 7. Sex composition of steelhead smolt samples in four size intervals.

Size interval (mm)	1981 M	1981 F	1984 M	1984 F	1985 M	1985 F
130–170	7	7	30	32	9	13
170–190	4	7	19	21	9	16
190–210	5	6	7	9	8	14
210–300	8	10	5	3	14	8
Sum	24	30	61	65	40	51
%	44.4		48.4		44.0	

for females where P_m = percent return of males as age .3, P_f = percent returns of females as age .3, and L = smolt length (millimetres).

If results of ageing male and female scales were combined into freshwater age groups 2, 3, 4, and 5, the percentage aged .3 within each group was 42.2, 31.4, 19.7, and 0%, respectively. This could be described linearly:

(5) $Pr = 96.24 - 0.36\,(L)$ ($r^2 = 0.99$, $p < 0.05$)

where Pr = percent returns of age .3 fish.

The biennial variation in age at return within steelhead runs (Table 1) was apparently a result of the variation in numbers and sizes of smolts. The dominant sea age class was .2 from smolt returns (males averaged 59% age .2, SD = 15; females 56%, SD = 12). Since smolt number and size varied biennially, a pattern of odd and even year class shifts thus appeared within runs.

TABLE 8. Age composition of male and female returns from steelhead smolts and the subsequent marine survival rates.

Smolt year	Ocean age: Males .1	.2	.3	Sum	Females .1	.2	.3	Sum	M and F	Smolts totals	Survivals
					Numerical composition						
1977	10	113	45	168		126	62	188	356	2 344	15.19
1978	1	40	40	81	0	48	27	75	156	2 104	7.41
1979	3	291	263	557	0	227	267	494	1051	6 900	15.23
1980	0	129	80	209	0	114	139	253	462	5 500	8.40
1981	67	1483	266	1816	2	877	323	1202	3018	11 897	25.36
1982	69	344	121	534	0	277	233	510	1044	4 000	26.09
1983	9	125	217	351	0	263	323	586	937	6 053	15.48
					Percent composition						
									Sex (% male)		
1977	6	67	27	100	0	67	33	100	47		
1978	1	49	49	100	0	64	36	100	52		
1979	1	52	47	100	0	46	54	100	53		
1980	0	62	38	100	0	45	55	100	45		
1981	4	82	15	100	0	73	27	100	60		
1982	13	64	23	100	0	54	46	100	51		
1983	3	36	62	100	0	45	55	100	37		
Mean	4	59	37		0	56	43		49		

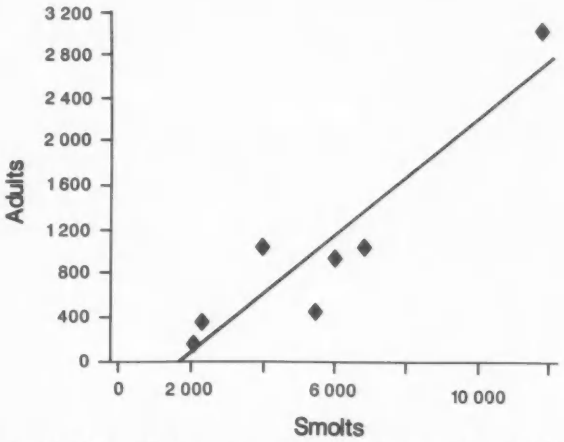

FIG. 3. Relationship between the number of emigrant smolts and adult returns from 1977 to 1983.

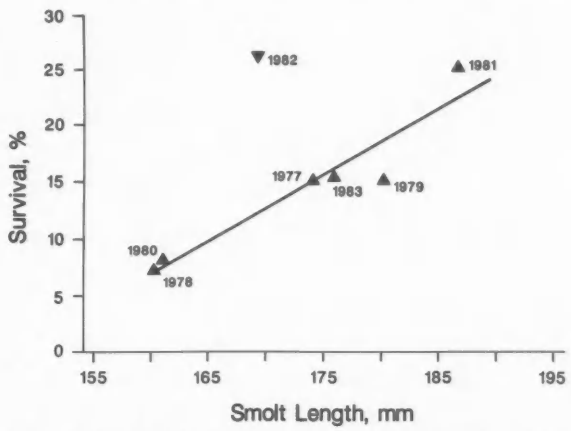

FIG. 4. Relationship between the marine survival of smolts and their mean length upon emigration.

The combination of the smolt number, the smolt size and survival relationship, and the smolt size at age and age at maturity relationship suggested a method of predicting run sizes. This was tested for the data used to develop these relationships.

Estimates of smolt returns based on freshwater age were calculated as

$$(6)\quad R = \sum_{i=2}^{4} n_i S_i$$

where R = adult returns from a cohort, i = freshwater age, n_i = number of smolts of age i, and S_i = survival rate as a function of length of age i smolts.

On average, prediction of survivors was overestimated by about 3%. As expected, 1982 smolt returns were underestimated, by 52%, but 1978 smolts were predicted to return 41% higher than measured by adult trapping and sampling. Some smolt lengths at freshwater age were outside the range of mean smolt lengths used to establish the size and survival relationship. The predictive equation remains speculative until the shape of the curve is defined to those limits.

Estimates of adults by age were calculated by including Pr:

$$(7)\quad R_3 = \sum_{i=2}^{4} n_i S_i \, \mathrm{Pr}_i/100$$

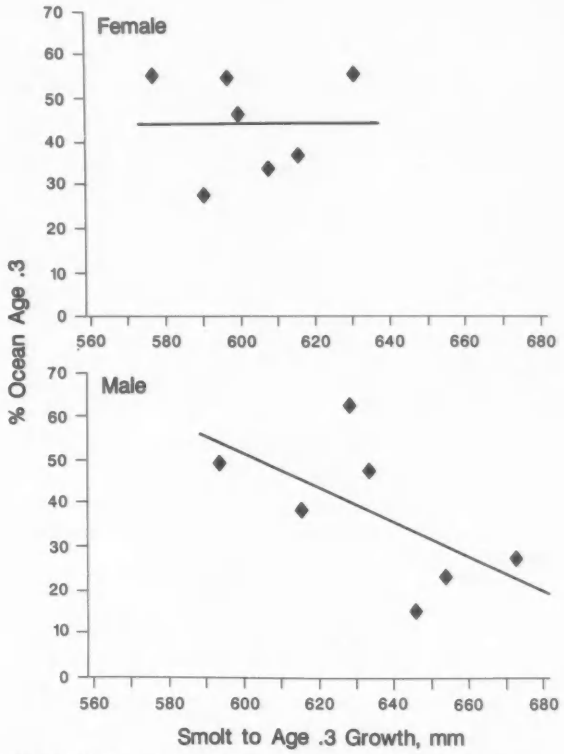

FIG. 5. Relationship between postsmolt growth to age .3 and return rate of age .3 (A) females and (B) males.

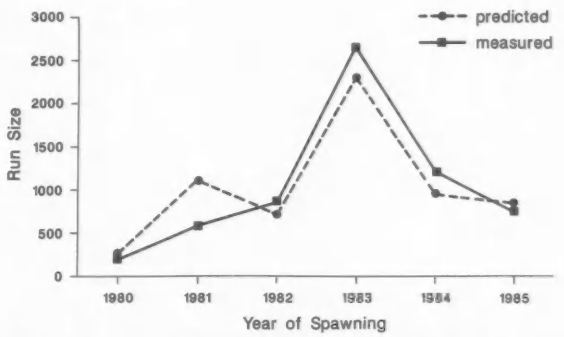

FIG. 6. Difference between steelhead run sizes (maiden fish only) as measured by mark–recapture and run sizes predicted by a model based on smolt size and number for the smolt returns spawning from 1980 to 1985.

$$(8)\quad R_2 = \sum_{i=2}^{4} n_i S_i (1 - \mathrm{Pr}_i/100)$$

where R_3 and R_2 are the returns at ages .3 and .2, respectively.

Calculated run sizes for the adult runs from 1980 to 1985 developed from equations 7 and 8 were compared with population estimates of maiden-run fish through the Keogh River trap, and agreement was fair. Calculated numbers were within 30% of run sizes, except for the adult run of 1981 which was overestimated 2.6 times that observed (Fig. 6).

Discussion

This study clearly indicated that run sizes of smolt and adult steelhead varied substantially between years (5- and 10-fold, respectively). Age structure also varied, accounting for changes in mean sizes of adults and smolts. Although based on a limited number of cohort returns ($n = 7$), adult numbers were directly proportional to smolt yield.

Much of the residual variation from the linear relationship of smolt and adult numbers was explained by smolt size. Mortality was highest for smaller smolts. There is increasing evidence that high mortality occurs early in marine life for sockeye (Peterman 1982; Ryall 1985), chinook (Neilson and Geen 1986), coho (Mathews and Buckley 1976), and chum fry (Healey 1982). These authors also noted size-dependent survival. This is supported by work on hatchery Atlantic salmon (Peterson 1972), hatchery coho (Bilton et al. 1982), and hatchery steelhead (Wagner 1967; Kenaston and MacHugh 1985) which indicated a positive relationship between hatchery smolt release size and subsequent return rate. Hager and Noble (1976) noted not only increased returns of hatchery coho with increased smolt size, but also found survival rates of male smolts slightly higher than female smolts. The relationship of smolt size and return rate may not be linear for smallest and largest smolts; the curve likely flattens at both ends.

Although smolt size (length) appeared to be the major causative variable, temperature changes in the marine environment were also probably important to marine survival. Smolts from 1982 survived two times higher than expected, according to the smolt size and survival relationship. Keogh hatchery smolts released in 1982 also survived about twice the average rates (data on file), and catches of steelhead by anglers on Vancouver Island in 1983–84 and 1984–85 (return years of 1982 smolts) were the highest on record (Billings 1986). This is supported by underwater surveys of Vancouver Island steelhead streams (M. G. Lirette, Ministry of Environment and Parks, Fisheries Branch, Nanaimo, B.C., pers. comm.). Mysak (1986) measured increased marine temperatures and noted numerous fish distributional changes associated with an El Niño event in 1982–83. We suggest that the marine events associated with El Niño may have resulted in improved survival for steelhead in the Vancouver Island region, but monitoring over a longer term is required to further examine the role of temperature in marine survival. Temperature effects in the marine environment and Atlantic salmon production near Iceland were positively related according to Scarnecchia (1984). In contrast, Johnson (1984) documented negative impacts of El Niño to coho and chinook salmon from the more southern latitude of Oregon.

The timing of the smolt migration may have also affected survival rates. The timing of the 1982 smolt migration was typical, but the peak migration time of the 1981 smolts was extended to a later second peak (data on file). Since the return data from the 1981 smolts had a large effect on results presented, some of the conclusions remain speculative until there is further investigation of the relationship of timing of wild smolt migrations and marine survival.

Age at Maturity

In Keogh River steelhead the relationship between freshwater age and ocean age was related to the size at freshwater age. Hooton et al. (1987) presented evidence that size at freshwater age or freshwater growth rate may have been related to jack returns but had little effect on older ocean ages of steelhead. Glebe and Saunders (1986) suggested that smolt size and subsequent growth rate rather than smolt age may be determining sea age at maturity in Atlantic salmon. Similarly, Hyatt and Stockner (1985) showed that increases in sockeye smolt size were correlated with earlier age at maturity. When individual smolt year classes were compared in the Keogh, no relationship existed between mean smolt size and return sea age. Either additive variability masked the relationship, scale samples for aging poorly represented the population, or the analysis was confounded by the effects of freshwater age and parental age.

Some of the variation in sea age for males may have been influenced by growth conditions at sea. Males might respond to rapid growth at sea by maturing earlier; because females varied less in size at age with varied growth conditions, they do not alter age at maturity. This suggests that age at maturity may be more stable in females whereas males are more plastic. In females, the threshold size or growth rate was possibly not great enough in the ocean to affect the age at maturity (Thorpe 1986). In addition, sea age may be under greater genetic control in females (Chadwick et al. 1986) than in males, and females may funnel surplus production elsewhere (fecundity or energy reserve). Growth and temperature effects in the ocean were inconclusive in relation to age at maturity of Atlantic salmon when summarized by Randall et al. (1986).

The effect of smolt size on sea age may vary in relation to parental sea age (Ritter et al. 1986). For females, growth at sea may be less important to age at return than the maternal age at maturity. Since Chadwick et al. (1986) found that ovarian development in female smolts was inversely related to sea age of parents, it follows that sea age of female smolts is largely determined at smoltification. Variation in sea age should nevertheless be expected within and between streams due to local adaptation and success or failure of various broods. As Myers (1986) suggested, some systems or conditions may favour large females, while others favour smaller, younger spawners. Smolts from such broods would vary from normal in parental sea age, confounding an analysis of smolt size effects on sea age due to the genetic component in age at return.

Changes in smolt number produced the biennial variation in ocean age structure. Large mean smolt size was followed by higher incidence of ocean .2's in the adult run, in general. Since males returned earlier than females, the males in odd-year runs (age .2) and the females in even-year runs (age .3) further exemplified the smolt size and number effect. The apparent termination of the biennial pattern (1985) coincides with a balancing of odd- and even-year pink salmon runs (Johnston et al. 1986) but may also be associated with hatchery fish returns or oceanographic conditions.

Adult size and age structure of Keogh River steelhead was typical of Vancouver Island steelhead populations (Hooton et al. 1987). Males were the largest and smallest adults in the population, as found with other salmonids (Gardner 1976). Most of the variation in adult steelhead size could be explained by the relationship of freshwater age and saltwater age.

Smolt Migrants

Variation in smolt size may be caused by the strong dominance of an even-year run of pink salmon (Johnston et al. 1986). Steelhead smolt sizes and number were highest following the even-year pink salmon cycle. Steelhead parr were observed intensively feeding on dislodged pink salmon eggs in the

autumn (data on file). Smolt length in odd years was greater than in even years and was related to freshwater age composition. Two different general shapes were apparent in length frequency distributions indicating not only larger mean size in odd years but also greater variation around the mean compared with smolt length in even years.

Mean smolt length was similar to that observed in other Vancouver Island streams (Hooton et al. 1987) and of wild smolts measured in Alsea River tributaries (Chapman 1958), but it was based on larger samples over a longer time period. Mean smolt size varies little on the Pacific coast (Withler 1966). Smolt length was limited to 13 and 30 cm, which was within the range of backcalculated smolt sizes predicted on the Chilliwack River (Maher and Larkin 1954). Hatchery fish released below (Wagner et al. 1963) or above (Partridge 1985; Slaney and Harrower 1981) the critical length remained in the stream, some for an additional year. Variation within the critical length between years and between streams probably relates to the age structure of the smolt population and stream production dynamics (Symons 1979).

Kelt Migrants and Repeat Spawners

The assumption of representative catch of kelts to derive the Petersen population estimates of adults was only tested and confirmed in one season of returns (winter of 1976–77). Fish capture may have depended largely on stream flow conditions during both spawner entry and kelt exit, and also on the genetic composition of the individual runs (Garrison and Rosentreter 1980). That is, a run comprised mainly from early-run fish may not have been as well represented in kelts compared with a run composed of relatively more late run fish. A similar problem may have existed for ocean age or adult size. It was also likely that kelt emigration was aided by relatively higher late winter and early spring stream flow, or conversely, impeded by low flow conditions. We assumed that these effects, if present, were negligible or equally affected each estimate.

Recapture rate of male kelts in 1983 was lowest, probably not only due to a relatively high composition of early run fish, but also because of extremely low discharge in May and June, impeding kelt movement downstream. However, even if the lower 95% confidence interval of the male estimate was used in the analysis presented, the resulting trends remained significant regarding smolt number, size, and adult returns. If the lower limit was used, the ocean age structure and sex ratios were affected. When examined by smolt cohort, this provided less variation than observed with the mark and recapture estimate. However, other observations, including redd counts and angler catch (data on file), supported the large population estimate of 1983. Further, the estimate of female adults in 1983 was known with more confidence. Since the difference in recapture rate between male and female kelts in 1983 was the same as other years, the male estimate was accepted, albeit with broad confidence intervals.

Composition of repeat spawners did not depend on the relative strength of the previous year's run. The ability for kelts to migrate out and survive an additional year at sea following spawning was probably the main determinant of repeat spawner composition. Frequency of repeat spawning in the Keogh River was similar to that observed from anglers' catch data elsewhere (Maher and Larkin 1954; Withler 1966). Similar rates of multiple spawning exist in Atlantic salmon populations (Schaffer and Elson 1975). Winter steelhead kelts were present incidentally in the commercial catch (Evans 1979), and this may vary in occurrence with seasonal openings or marine environmental conditions. Differences in flow patterns and catch plus variation in kelt health between years may have altered repeat spawning rates.

Management and Research Implications

The large variation in adult returns of Keogh winter run steelhead provides a clear indication of the problem fisheries managers face in managing steelhead fisheries. Inability to predict effects of fluctuating smolt rearing conditions, smolt size and age changes, and marine conditions has been largely responsible for conservative management strategies in British Columbia. Adult population estimates for the Keogh River demonstrated for the first time the order of magnitude of fluctuation possible in a wild steelhead population where fishery pressure was low.

Another productive aspect of these ongoing studies has been identification of areas requiring more research. Future research should focus on the smolt sex ratio within size intervals (and in relation to hatchery grading practices). The marine survival of males versus females should be compared. Smolt sizes at age compared with adult scale backcalculated sizes at age and backcalculated sizes at age .1 in relation to return age should also be examined. The role of smolt size or age and the age at return will remain uncertain for steelhead until interactions between environmental and genetic factors are better understood. The effect of smolt size on marine survival of steelhead needs to be verified and examined further over a longer time series with tagged smolts sampled over the migration period, and in broader ecological settings. The eventual establishment of predictive models of returns based on smolt number, size, and other characteristics (e.g. sex) may greatly assist the understanding and management of steelhead populations.

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Morphological Deformities in *Chironomus*, *Cryptochironomus*, and *Procladius* Larvae (Diptera: Chironomidae) from Two Differentially Stressed Sites in Tobin Lake, Saskatchewan

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Warwick, W. F., and N. A. Tisdale. 1988. Morphological deformities in *Chironomus*, *Cryptochironomus*, and *Procladius* (Diptera: Chironomidae) from two differentially stressed sites in Tobin Lake, Saskatchewan. Can. J. Fish. Aquat. Sci. 45: 1123–1144.

Morphological deformities in *Chironomus plumosus* type larvae from two differentially stressed sites in the Tobin Lake reservoir are described and compared. The frequency of deformities was 41.3% at Site 2 where larvae are directly exposed to contaminants entering the reservoir from the Saskatchewan River. At Site 17, where larvae are only indirectly exposed in a backwater area, the frequency was 31.8%. The frequency of deformities was higher at Site 2 in all head capsule structures except antennae. At Site 17, the significantly higher frequency of antennal deformities suggests that (1) antennae respond to contaminants according to a quantal dose–response relationship, (2) antennae and other head capsule structures may respond through different mechanisms or at different rates, or (3) other environmental factors may modify larval responses to contaminants. Descriptions of the deformities in other head capsule structures provide a framework for individual indices of severity of deformation similar to that developed previously for antennae. These eventually will be incorporated into an Index of Total Morphological Response. Deformities in *Procladius* and *Cryptochironomus* larvae are also described.

Les auteurs décrivent et comparent les anomalies morphologiques de larves de l'espèce *Chironomus plumosus* provenant de deux emplacements du réservoir du lac Tobin perturbés de façon différente. La fréquence des anomalies a été de 41,3 % à l'emplacement 2 où les larves sont exposées directement aux contaminants apportés dans le réservoir par la rivière Saskatchewan. À l'emplacement 17, où les larves ne sont exposées qu'indirectement, se trouvant dans un secteur d'eaux dormantes, la fréquence a été de 31,8 %. La fréquence des anomalies de toutes les structures de la capsule céphalique à l'exception des antennes a été plus élevée à l'emplacement 2. À l'emplacement 17, la fréquence significativement plus élevée des anomalies des antennes donne à penser 1) que les antennes réagissent aux contaminants suivant une réaction positive ou nulle en fonction de la dose, 2) que les antennes et d'autres structures de la capsule céphalique peuvent réagir par des mécanismes différents ou à des taux différents, ou 3) que d'autres facteurs environnementaux peuvent modifier les réactions des larves aux contaminants. Des descriptions d'anomalies touchant d'autres structures de la capsule céphalique offrent la possibilité de déterminer des indices individuels de la gravité des déformations semblables à ceux établis précédemment pour les antennes. Ces descriptions seront intégrées éventuellement dans un indice de réaction morphologique totale. Les auteurs décrivent également les anomalies rencontrées chez les larves de *Procladius* et de *Cryptochironomus*.

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Although contaminants pose one of the most serious environmental problems facing society today, their chemical characteristics and probable impact can seldom be determined because of the complexity and expenditure involved. Thus, emphasis is shifting towards biological methods for assessing the severity of contamination. However, as yet there are few, if any, widely accepted and reliable methods available for detecting and assessing the impact of contaminants on biological communities residing in, and depending on, freshwater ecosystems (National Research Council of Canada 1985).

Warwick (1985) proposed using morphological deformities in chironomid larvae as a biological screening tool to detect and assess the impact of contaminants in freshwater ecosystems. Hamilton and Saether (1971) linked morphological deformities to contaminants because deformed larvae generally were found only in areas receiving industrial and/or agricultural contaminants, but not in areas polluted by domestic sewage. Subsequent observations by a number of authors (Saether and McLean 1972; Hare and Carter 1976; Warwick 1980a, 1980b, 1980c; Köhn and Frank 1980; Tennessen and Gottfried 1983; Wied-

erholm 1984; Cushman 1984; Warwick et al. 1987) support the Hamilton/Saether hypothesis. Warwick (1985) and Percy et al. (1986) presented experimental evidence confirming the linkage between contaminants and morphological deformities in chironomid larvae.

Warwick (1985) stressed the need to develop a framework within which the severity of morphological deformities could be assessed and used to gauge response. As a first step, he proposed a quantitative numerical index based on deformities in the antennae of larvae of *Chironomus* Meigen. The objectives of this paper are to (1) document the types of deformities in *Chironomus plumosus* type larvae from two differentially stressed sites in Tobin Lake, (2) explore the range of severity in these deformities and compare their distributions in the two faunas, (3) establish a framework for indexing the severity of deformation in additional morphological structures for eventual inclusion in an overall Index of Total Morphological Response for *Chironomus* larvae, and (4) begin similar documentation for *Procladius* and *Cryptochironomus* larvae.

Study Area

The materials used in this study came from Tobin Lake, east-central Saskatchewan (Fig. 1). Tobin Lake is a reservoir on the mainstem Saskatchewan River formed in 1960–63 by impoundment behind the Squaw Rapids hydroelectric dam. The reservoir is made up of two distinct basins: one parallels the original Saskatchewan River valley and the other includes the original Petaigan River valley and some bordering agricultural land. The greater part of the contaminant burden to the lake enters via the Saskatchewan River system. This includes agricultural and industrial residues from the greater part (270 000 km²) of the Canadian prairie provinces. The drainage basin of the Petaigan River is much smaller (466 km²), includes only agricultural lands, and probably contributes only a very small proportion, if any, of the contaminant burden to the reservoir. The exchange of waterborne materials between the two basins probably is limited by basin morphometry (Fig. 1). Heavy *Typha* beds also screen the two basins except where the deepwater channel connects.

Chironomid larvae were collected from two sites: one in each basin for purposes of comparison. Tobin Lake Site 2 is located (53°28′44″N, 103°50′51″W) in the deposition zone just below the inflow of the Saskatchewan River (Fig. 1). Benthic communities in this area are directly exposed to contaminants borne by the Saskatchewan River. Tobin Lake Site 17 is located (53°33′16″N, 103°28′16″W) in the former Petaigan River valley. Site 17 is influenced more by the intermittent flow of the Petaigan River than backflow from the main Saskatchewan system. Both sites were identified as primary sites for chemical, physical, and bioassay analysis in the original Tobin Lake project proposal (Birkholz et al. 1980).

Methods and Materials

Tobin Lake Sites 2 and 17 were sampled for benthos, water, and sediments on 8 and 15 February 1980. Each benthic sample included the contents of three tall 232-cm² Ekman grabs. The samples were sieved through 200-μm-mesh nets of Nitex nylon within 24 h of collection. Materials retained in the sieve were preserved in 70% ethanol, stained with Rose Bengal, and sorted. Sorted benthos were separated into individual taxa, enumerated, and preserved in fresh 70% ethanol for future analysis.

Preserved chironomid larvae were prepared and mounted in Canada balsam according to the methods described in Warwick

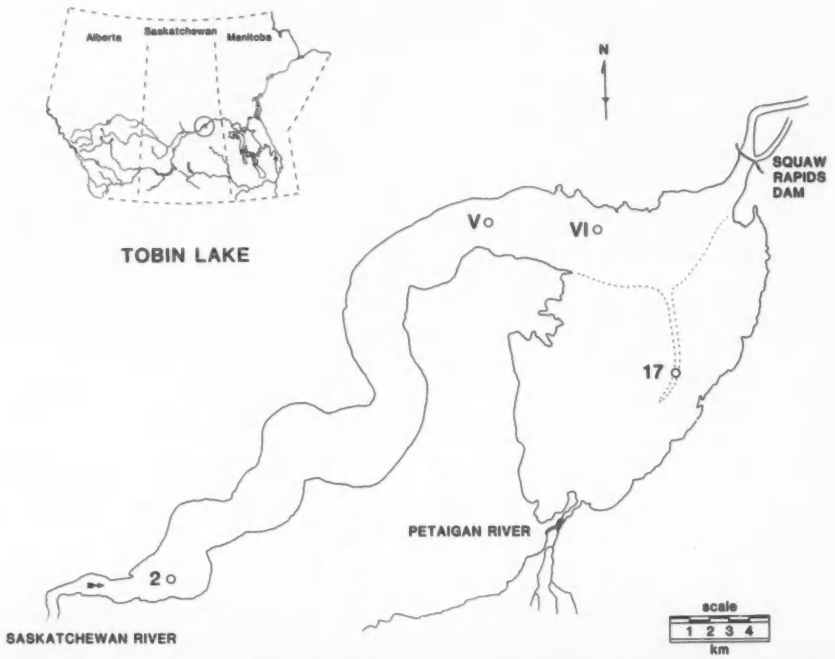


FIG. 1. Location of sampling sites in the Tobin Lake reservoir, February 1980.

TABLE 1. Comparison of chironomid faunas from Tobin Lake Sites 2 and 17.

	Site 2		Site 17	
	No.	%	No.	%
Chironomus plumosus type	482	41.5	153	31.4
Chironomus thummi type	5	20.0	0	0.0
Chironomus salinarius type	0	0.0	1	100.0
Procladius (Holotanypus) sp.	259	25.6	77	24.9
Procladius (Psilotanypus) bellus (Loew)	31	3.1	1	0.3
Procladius spp.	54	5.4	19	6.1
Cryptochironomus sp.	123	12.2	45	14.6
Other taxa	56	5.5	13	4.2
Total	1010		309	

(1980a, 1985). These methods permit the examination of fine morphological detail using high-power, oil-immersion objectives. No attempt was made to identify larvae to species because of the problems involved in the identification of chironomid immature stages (cf. Lindeberg and Wiederholm 1979).

In this study, morphological deformities were found in the antennae, mentum, mandibles, premandibles, epipharyngeal pecten, labral lamellae, and labral setae. These deformities were categorized according to the following framework: (1) *antennal deformities* according to (a) the loss or fusion of individual segments, (b) the loss or displacement of auxilliary sensory structures, (c) reduction in the length of any or all segments, and (d) the presence of structures of unknown equivalence following Warwick (1985); (2) *mentum deformities* occurring in (a) the tripartite median teeth, (b) the two large, inner lateral teeth, (c) the four smaller, outer lateral teeth, and (d) those occurring generally throughout the mentum or (e) showing asymmetry or massive disorganization of the basic configuration; (3) *mandible deformities* involving (a) the number of subapical or apicodorsal teeth, (b) the conformation of the apical tooth, (c) the presence, form, and pattern of the spurs, setae, and pecten and (d) the general configuration of the mandible; (4) *premandible deformities* involving (a) the number of apical teeth, (b) the configuration of individual teeth, and (c) the general configuration of the premandible; (5) *deformities in the epipharyngeal pecten* showing (a) fusing, (b) overlapping, (c) stunting, and (d) disjunction of the epipharygeal structure; (6) deformities in the *labral lamellae* involving (a) division along the median suture line, (b) reduction in size (in whole or in part), (c) malformation of individual lamella, and (d) duplication and overlapping of individual sets of lamellae; and (7) deformities in *labral setae* involving departure from the normal plumose configuration.

The frequency of deformities was determined as the ratio of the number of actual to possible occurrences expressed in percent. The term "frequency" (sensu "prevalence"; Margolis et al. 1982) replaces the term "incidence" used in Warwick (1985, 1988).

The severity of antennal deformations was assessed according to Warwick (1985); no attempt has been made at this time to assess the severity of deformities within the framework proposed for the other structures outlined above.

Results

Chironomid Community Comparisons

The chironomid communities at Sites 2 and 17 were dominated by *Chironomus*, *Procladius*, and *Cryptochironomus* larvae (Table 1). The highest frequencies of deformities were in *Chironomus* larvae. At Site 2, 41.3% of *Chironomus* larvae were deformed compared with 31.8% at Site 17. The frequencies of deformities in *Procladius* and *Cryptochironomus* larvae were an order of magnitude less. In *Procladius* larvae, the frequencies of deformities were 4.4 and 2.1% at Site 2 and 17, respectively, and in *Cryptochironomus* larvae were 4.9 and 6.7% at Site 2 and 17, respectively. The data for *Cryptochironomus* should be regarded as tentative at this time because the information available to interpret the response of this genus is still very limited.

A comparison with the frequency of deformities in a chironomid population of very similar composition from Last Mountain Lake, south-central Saskatchewan, is useful to set these results in context. Last Mountain Lake Site I is located in the southern end of Last Mountain Lake and is contaminated by river water shunted back up into the lake from the Qu'Appelle River system during spring flooding (Warwick 1985). These events are periodic and usually of short duration when they do occur. In Site I, the frequencies of deformities in *Chironomus*, *Procladius*, and *Cryptochironomus* larvae was 16.2, 1.5, and 0.8%, respectively (W. F. Warwick, unpubl. data). Compared with this mildly contaminated site, the frequencies of deformities were more than twice as high in most cases at both Tobin Lake sites.

Also by way of comparison, Warwick (1985) reported that the frequency of deformities in cultures of *Chironomus tentans* Fab. held under controlled laboratory condition was 1–2%. Similar frequencies were recorded from populations of *C. riparius* maintained under laboratory conditions (Frank 1983).

Description of Morphological Deformities in *Chironomus plumosus* Type Larvae

1. Normal and deformed antennae

The five-segmented antenna of normal *Chironomus* spp. larvae (Fig. 2a) is a complex sensory organ bearing multiple sensillae which serve in mechano- and chemoreceptor roles. The conjoined blade and accessory blade and the peg sensillae probably are chemoreceptors and the ring organ, basiconic sensillum, style, and organs of Lauterborn probably serve as mechanoreceptors. The function of these receptors was discussed in detail in Warwick (1985).

The antennal deformities found in Tobin Lake Sites 2 and 17 display much the same range of deformities described in Warwick (1985). The more obvious deformities involve the loss of individual segments (Figs. 2d, 2f), the fusion of one or more adjacent segments (Fig. 2c), or the presence of segments of questionable equivalence (Figs. 2c, 2e, 2g). The antenna in Fig. 2b is unusual in having an additional (6th) segment appended along the side of the penultimate (4th) segment. Individual antennae in many cases are reduced in length; these reductions can occur generally or be confined to basal, distal, or individual segments (Fig. 2f). In some cases, the ring organ may be displaced or entirely lacking. Other sensory organs such as the blade/accessory blade complex or the style may be displaced from their normal positions at the apices of the first (Fig. 2h) or second segment, respectively. In some cases, the apical segments are lost (Figs. 2i, 2j) and only remnants of the sensory structures remain (Figs. 2k, 2l) or the apices of the basal segment are completely fused over (Fig. 2m). In more unusual cases, structures appear for which no homology is known. These may range from simple protuberances to complex trident- or palmate-shaped structures (Warwick 1985).

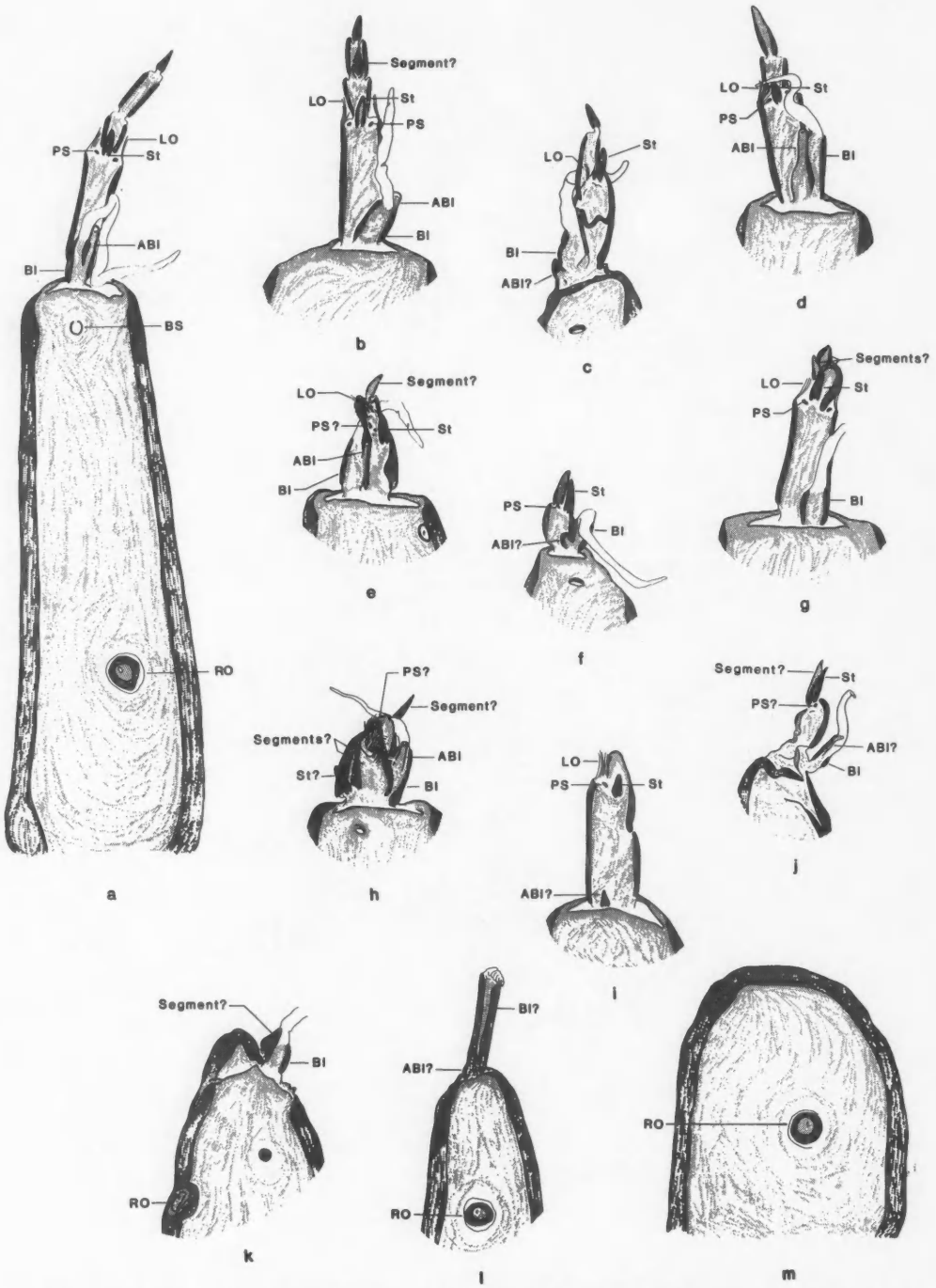


FIG. 2. Deformities in antennae of *Chironomus plumosus* type larvae: (a) normal antenna; (b–m) deformed antennae. Bl, blade; ABl, accessory blade; St, style; PS, peg sensillum; LO, organs of Lauterborn; BS, basiconic sensillum; RO, ring organ.

2. *Normal and deformed menta*

The mentum is a double-walled medioventral plate (Fig. 3a) consisting of the dorsomentum and ventromentum in which the latter extends laterally into the ventromental plates (Saether 1980). The mentum has 15 darkly pigmented teeth made up of a tripartite median arrangement of one large tooth flanked on either side by single smaller teeth and two larger and four smaller outer teeth on either side. The heavily pigmented pat-

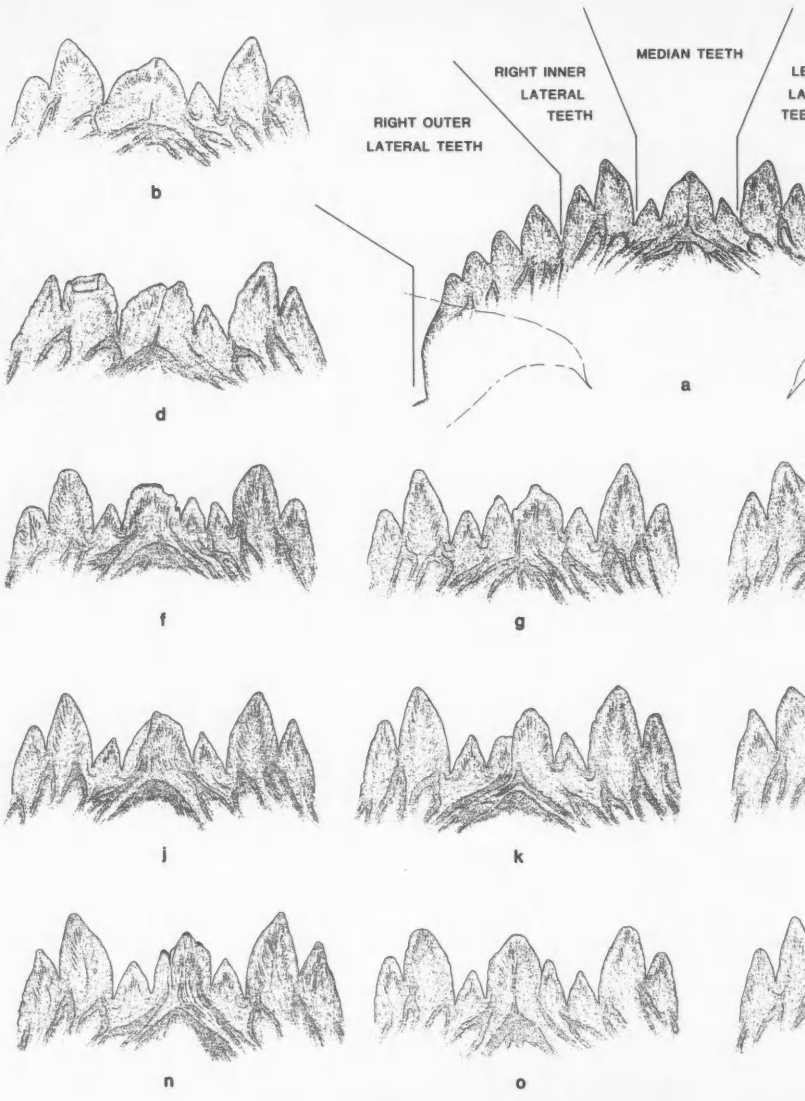
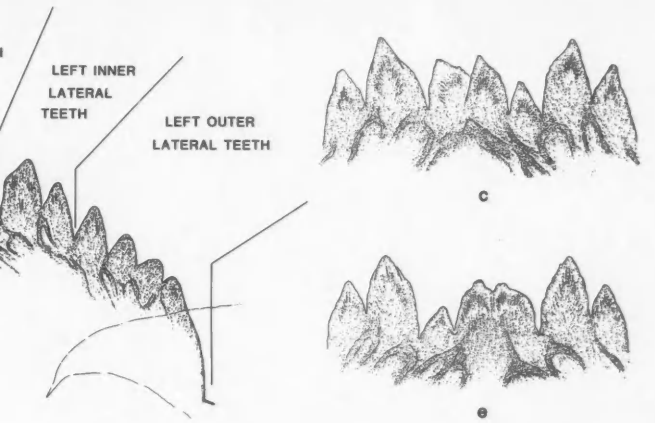


FIG. 3. Deformities in the median teeth of the mentum of *Chironomus plumosus* type lar



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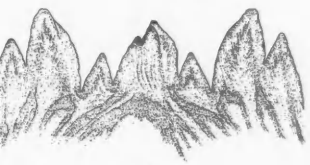

h

i

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type larvae: (a) normal median teeth; (b–q) deformed median teeth.

terns extending below the teeth suggest areas of thickened chitinous reinforcement for the teeth. The ventromenta of *C. plumosus* type larvae are characteristically marked by a curved row of fascia-like bundles of stria located just below the anterior margin.

Deformities in the mentum can be divided into five basic categories: (a) those occurring in the three median teeth, (b) those occurring in the two larger, inner lateral teeth, (c) those occurring in the four smaller, outer lateral teeth, (d) those occurring generally throughout the mentum, and (e) those showing asymmetry or massive disorganization in basic configuration.

(a) Median deformities — The number of teeth in the median area of the mentum can vary from the normal tripartite arrangement to menta with two, three, four, and five median teeth (Fig. 3). An example with two teeth is shown in Fig. 3b. In this case, one of the smaller side teeth is missing. The examples in Fig. 3c and 3e have three teeth, but one of the smaller side teeth is enlarged. The example in Fig. 3d might be mistaken for three teeth, but the pattern of stria on the teeth indicates that the larger tooth is split rather than a third tooth being present as in Fig. 3e. Examples with four and five median teeth are shown in Fig. 3f–3i. In Fig. 3i, the extra teeth are represented by two tiny teeth symmetrically sited submarginally at the junction of the median and first lateral teeth. As many as eight median teeth have been found in deformed larvae from other sites (Warwick 1980c).

The remaining examples in Fig. 3 show some of the range of variability that can occur in median areas having four teeth. The separation of the fourth tooth becomes progressively more apparent between Fig. 3j, where the smaller central tooth overlaps the larger tooth, and Fig. 3o, where each tooth is well separated. The additional tooth may also be quite small and located at the base (Fig. 3p) or on the shoulder (Fig. 3q) of the central tooth.

The central teeth in the menta in Fig. 4 are reduced in size (Fig. 4b, 4c), malformed (Fig. 4d, 4e), and split (Fig. 4f–4i). In the classical sense, splitting in the median tooth usually is symmetrical (Fig. 4h). In specimens from other sites, examples have been found where splitting has penetrated to the base of median tooth (W. F. Warwick, unpubl. data). The divergent pattern of stria on these teeth shows that they represent single teeth that have been split rather than two separate teeth. The mechanism of formation clearly differs from that producing the extra median teeth shown in Fig. 3.

Although less frequently observed, a number of central teeth displayed (Fig. 4j–4l) the crusting typical of larvae from lakes forming the Southern Indian Lake–Notigi Reservoir system which were affected by mercury contamination (Bodaly et al. 1984). These encrustations appear as small darkened areas along the outer margins of the teeth. The margins of these teeth wear unevenly suggesting that the darkened areas are harder than the surrounding lighter areas. This is particularly apparent in Fig. 4l. The central tooth in this illustration is also deeply split.

A number of specimens displayed characteristic "Köhn" gaps in the median area of the mentum. These gaps were characteristic of *Chironomus thummi* larvae collected from the heavily polluted Teltowkanal in Berlin, Germany (Köhn and Frank 1980). True gaps can be identified by the lack of colour and support ribbing underlying the area of the gap itself. The presence of the gap may (Fig. 4o) or may not (Fig. 4m, 4n) involve the loss of one or more of the tripartite median teeth.

Several larvae had characteristic "keyhole" openings in the central tooth (Fig. 4p, 4q). Similar, but somewhat more bizarre specimens of this type have been found in larvae from Crooked Lake, Saskatchewan (W. F. Warwick, unpubl. data).

(b) Inner lateral deformities — The number of inner lateral teeth may be increased (Fig. 5b, 5c) or reduced (Fig. 5d) to single teeth. The two lateral teeth can also be incompletely separated (Fig. 5e–5g) to form bifid first lateral teeth. Intermediate examples can intergrade to the point where bifid first laterals and those with three inner lateral teeth can be difficult to separate.

(c) Outer lateral deformities — The number of outer lateral teeth may also be increased (Fig. 5h) by additional teeth or reduced to three (Fig. 5j, 5i) or two (Fig. 5l, 5k) outer lateral teeth. Examples with only one outer lateral tooth have not yet been found, but the number of additional teeth can achieve startling proportions; larvae with 39 (total 48) and 48 (total 57) additional teeth have been found in samples from Tobin Lake and the former Rat Lake basin of the Southern Indian Lake – Notigi Reservoir, respectively (W. F. Warwick, unpubl. data).

Examples of additional teeth in the form of small, sublaterally mounted teeth were also found (Fig. 5m–5o). In contrast with the additional tooth in Fig. 5h which was full-sized and in line with the other outer lateral teeth, these teeth were less well-formed, occurred singly (Fig. 5m, 5n) or in pairs (Fig. 5o), and were variously positioned along the outer lateral margin of the mentum. Similar rudimentary teeth were noted above (Fig. 3i) in the central area of the mentum.

Teeth can also overlap one another. In the outer lateral teeth, overlapping usually occurs between the fourth, fifth, and sixth teeth (Fig. 5p, 5q), but other combinations have been found. Classic overlapping is readily distinguished from some normal specimens in which the fifth tooth tends to appear separate.

(d) Integral deformities — These refer to deformities that are not identified with specific areas of the mentum, but apply to the mentum in general. Structural weakening of the mentum shown by the lack of colour and wearing in individual teeth (Fig. 5r–5u) would fall into this category. The three outer teeth in Fig. 5r are unusual in that they are almost colourless and yet are intact. Usually such teeth are badly worn (Fig. 5s) or worn away completely (Fig. 5t). The latter can be difficult to distinguish from normal teeth that have been broken by other means, but usually there is enough of the light-coloured area remaining to make the determination reliably. If there is not, the conservative approach would be to disregard the specimen as broken by natural causes. Teeth of this type can appear in the inner lateral (Fig. 5u) or central (Fig. 4r) teeth as well.

The interpretation of poor structural strength is based on data from preliminary dose–response experiments using DDE (W. F. Warwick, unpubl. data). *Chironomus tentans* larvae with colourless menta were found in cultures exposed to 10 μg DDE·L^{-1}. The granular, sugar-like texture of these menta gave little impression of strength and all were badly worn.

(e) Basic Configuration — The deformities discussed independently above can appear in combinations of increasing severity (Fig. 6a–6l) which can lead to individuals whose menta bear little resemblance to the basic configuration. For example, the mentum in Fig. 6a has four median teeth and one inner lateral tooth. The example in Fig. 6b is similar, but the configuration of the median teeth differs slightly. The mentum in Fig. 6c also has four median teeth, but only three outer lateral teeth are present. In Fig. 6d, the mentum has four median teeth, one pale-coloured outer lateral tooth, and one smaller, subla-

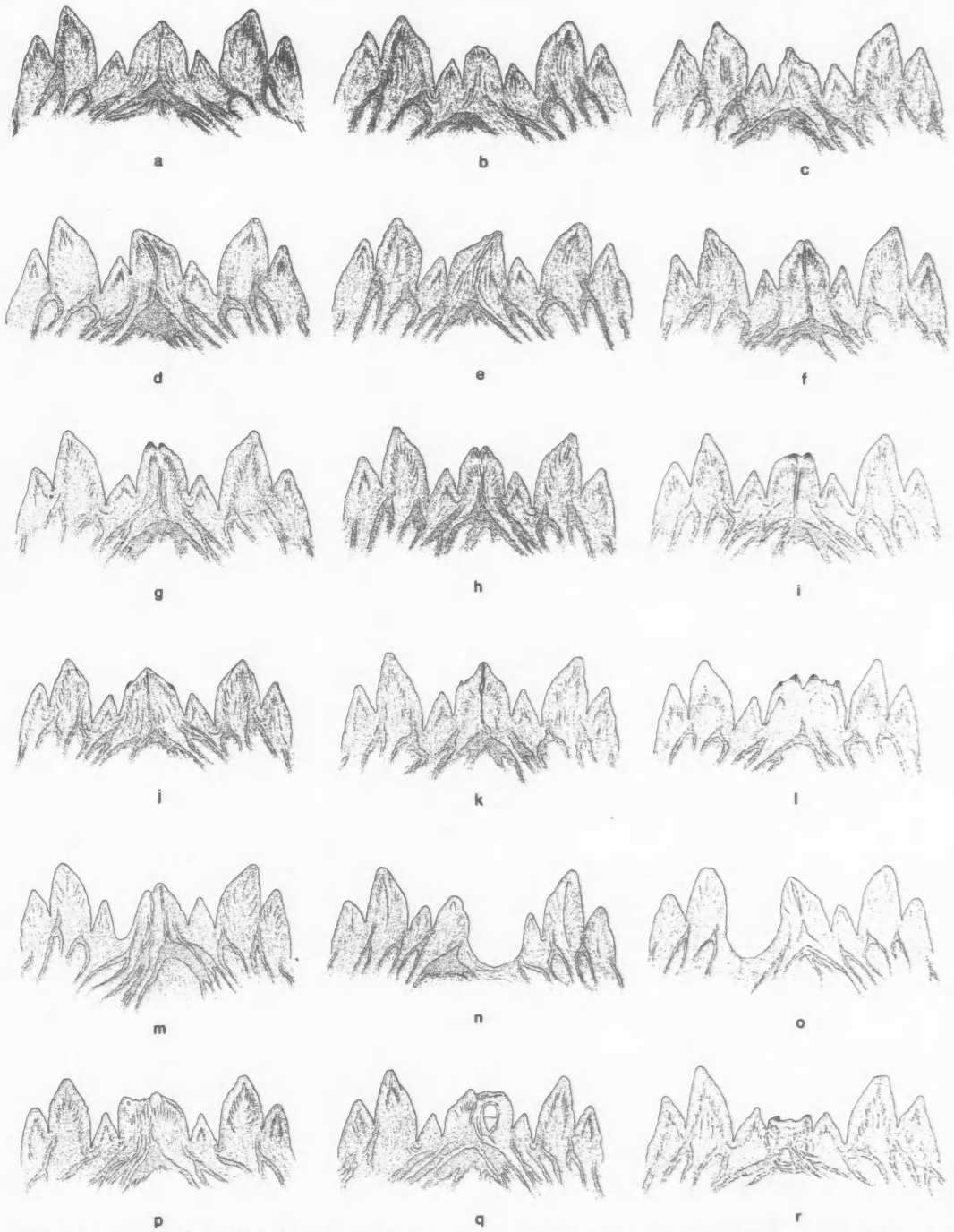


FIG. 4. Deformities in the median teeth of the mentum of *Chironomus plumosus* type larvae: (a) normal median teeth; (b–r) deformed median teeth.

terally mounted tooth. The example in Fig. 6e has three lateral teeth on the right side of the mentum and only single inner lateral teeth, the one on the right of which is considerably enlarged. The central tooth in Fig. 6f is split, one inner lateral (left) is missing, and both large inner lateral teeth are bifid. The example in Fig. 6g has four median teeth, a single bifid inner lateral tooth, and evidence of some asymmetry on the left side. The asymmetry is more pronounced in Fig. 6h where the men-

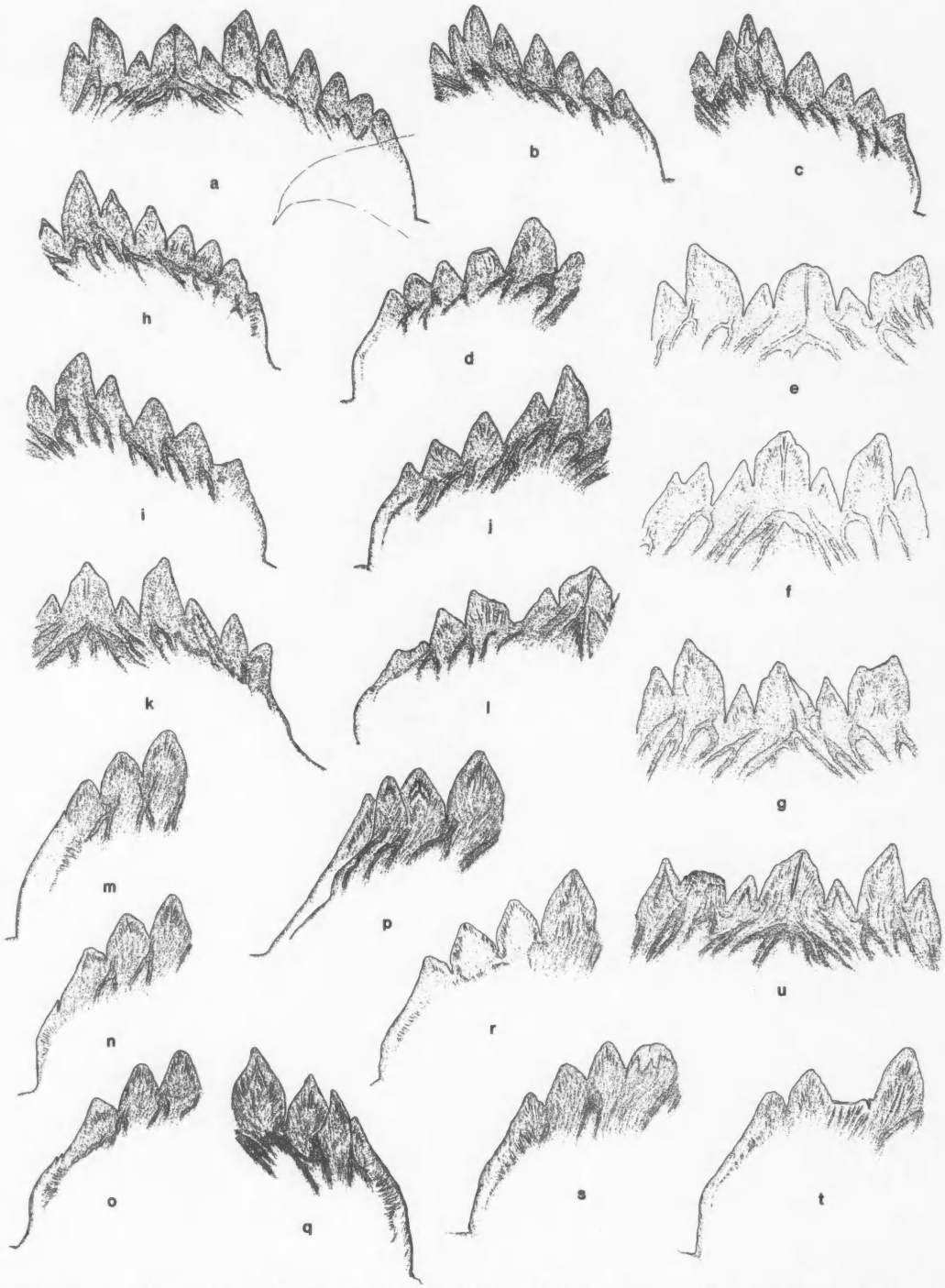


FIG. 5. Deformities in inner and outer lateral teeth of the mentum of *Chironomus plumosus* type larvae: (a) normal inner and outer lateral teeth; (b–u) deformed inner and outer lateral teeth.

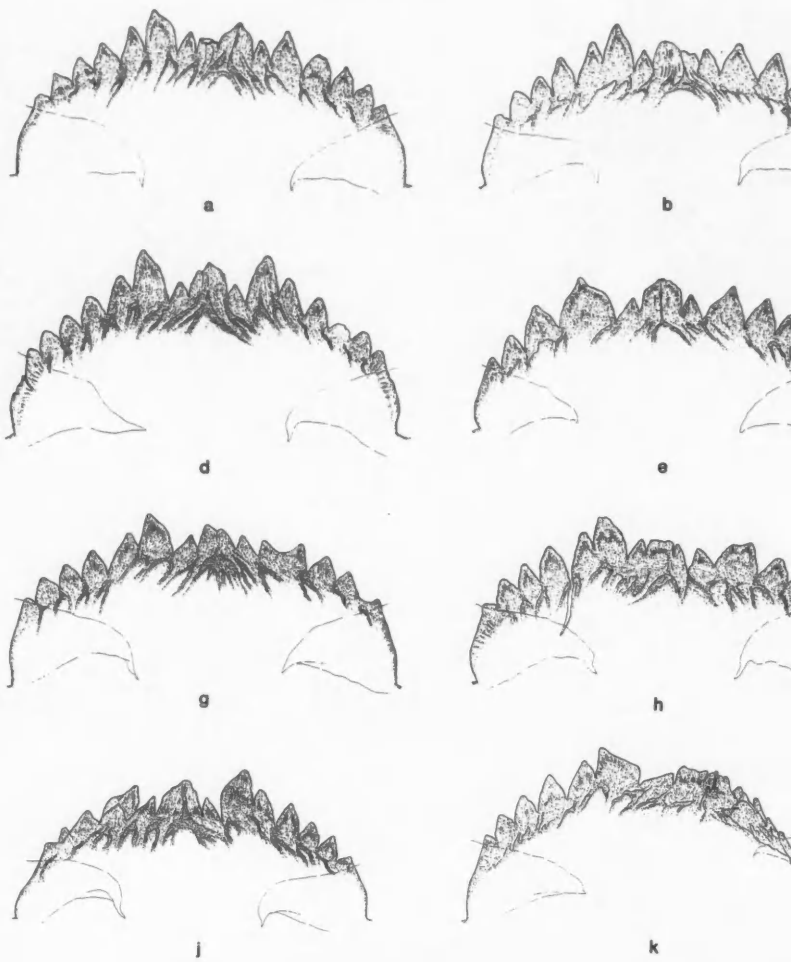
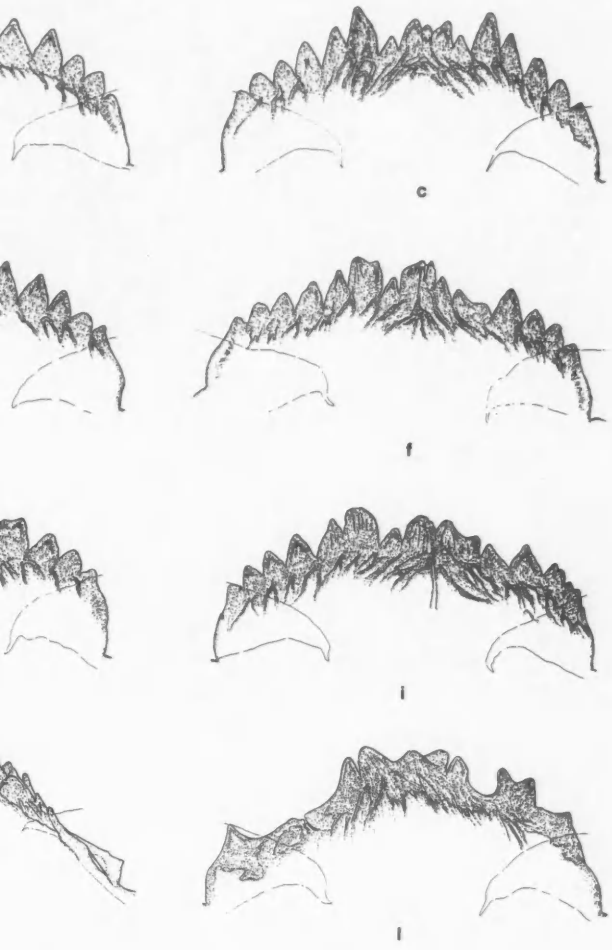


FIG. 6. Deformities in the menta of *Chironomus plumosus* type larvae: deformed menta



d menta displaying various combinations of deformity types.

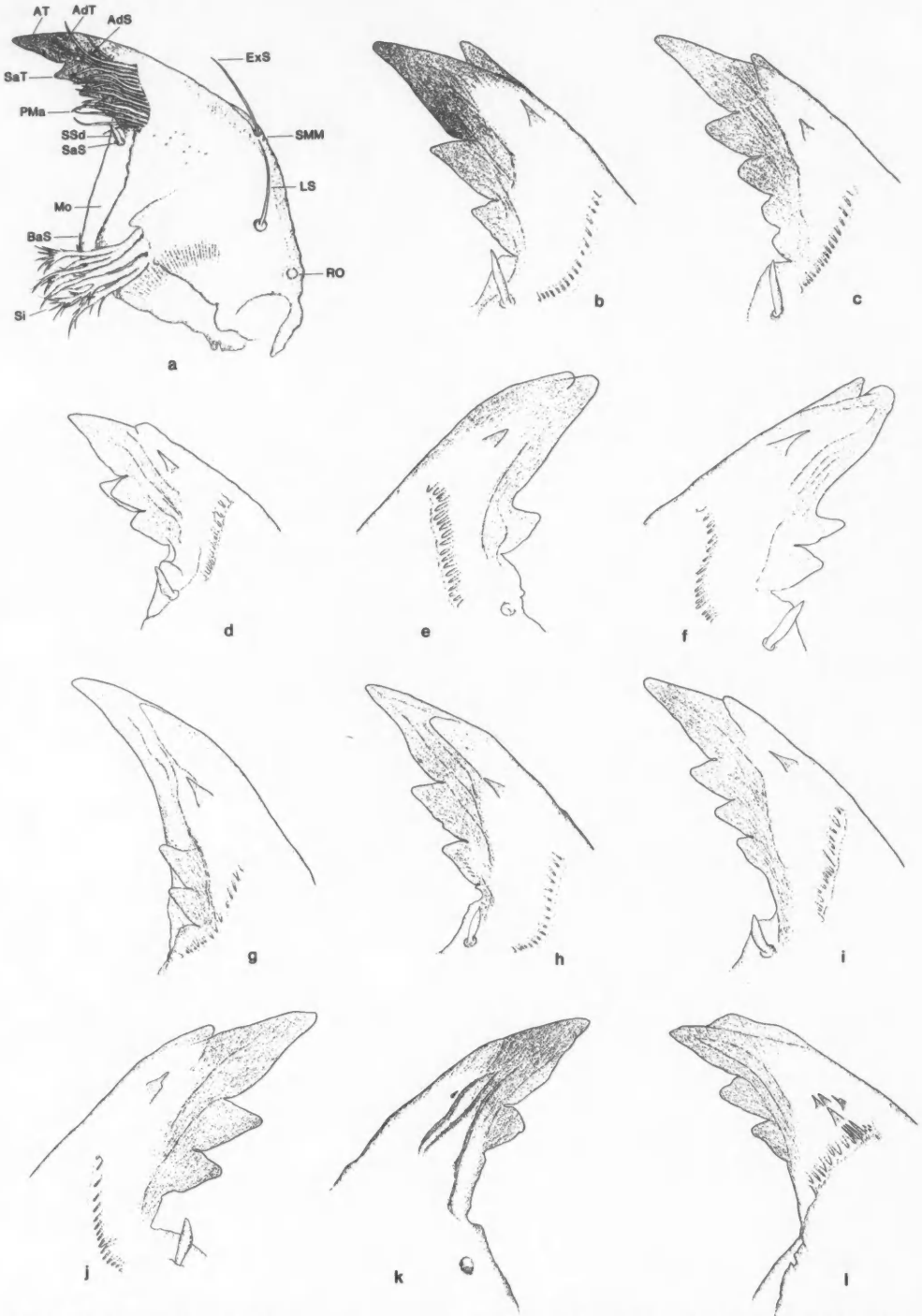


FIG. 7. Deformities in the mandibles of *Chironomus plumosus* type larvae: (a) normal mandible; (b–l) deformed mandibles. AT, apical tooth; AdT, apicodorsal tooth; AdS, apicodorsal spur; ExS, external seta; SMM, sensillum minusculum; LS, lateral seta; RO, ring organ; Si, setae interna; BaS, basal spurs; Mo, mola; SaS, subapical spurs; SSd, seta subdentalis; PMa, pecten mandibularis; SaT, subapical teeth.

tum has four median teeth, a single enlarged inner lateral tooth, and three outer lateral teeth. The left side of the mentum in Fig. 6i is more seriously deformed; there is one sublateral tooth, five overlapping outer lateral teeth, one inner lateral tooth, and four median teeth, one of which is bifid and another of which is superimposed. The right side of the mentum in Fig. 6j has four lateral teeth which not only overlap in themselves, but also overlap the single inner lateral tooth. The central tooth is also mildly asymmetrical. The mentum in Fig. 6k is severely deformed; there are 14 teeth representing the left-medial part of the mentum for a total complement of 20 teeth. The mentum in Fig. 6l is so severely deformed that verbal descriptions become quite inadequate.

3. Normal and deformed mandibles

The mandibles of normal larvae are heavily sclerotized structures (Fig. 7a) located laterally on either side of the mouth. The apical half is usually attenuated with an apical tooth, three subapical or inner teeth linearly along the inner mesal margin, and a single dark apicodorsal or outer tooth on the outer margin. On *C. plumosus* type larvae, all mandibular teeth characteristically are dark brown to black. One, sometimes two, and, infrequently, three dark spurs are located inwardly below the apicodorsal tooth, while the blade-like seta subdentalis arises immediately below the inner teeth of the mandible. The pecten mandibularis, a comb of stiff, straight setae, angles distad across the distal mesal side of the mandible. The setae interna, a tuft of (usually three) branched setae, is located on the inner dorsomedial side below the mola, a weakly sclerotized area of the mandible. One to three sharply hooked basal spurs, often masked by the setae interna, are located on the ventromedial corner of the mandible. A large ring organ, probably a mechanoreceptor, is located ventrolaterally at the base of the mandible. One or two subapical spurs occur at the base of the seta subdentalis. In *C. plumosus* type larvae, these are small and bluntly triangular compared with the longer, more pronounced spurs in other *Chironomus* type larvae. The sensillum minusculum is a tiny sensilla located ventrolaterally on the mandible just lateral to the long external seta near the angle of the convex edge. The mandible probably functions in food gathering.

Mandible deformities generally involved the numbers of subapical and apicodorsal teeth, conformation of the apical tooth, the presence, form, and pattern of the spurs, setae, and pecten, and the general morphology of the mandible. Compared with the normal configuration (Fig. 7a), the mandibles in Fig. 7b and 7c have two and four subapical teeth, respectively. The mandible in Fig. 7d has three subapical teeth, but two are superimposed rather than on the same plane. Only one subapical tooth remains on the mandible in Fig. 7e; the apical tooth in this example appears proportionately longer, possibly because only one subapical tooth remains, but the longer apical teeth in the mandibles in Fig. 7f and 7g, each of which have two subapical teeth, suggest that the apical tooth can undergo a process of elongation. In contrast, the subapical teeth may also move distad rather than basally to leave a progressively wider gap between the base of the subapical teeth and the medial margin. This is clearly shown in Fig. 7h–7l. The same phenomenon is evident whether three (Fig. 7i), two (Fig. 7h, 7j, and 7l), or only one (Fig. 7k) subapical tooth remains.

The more strongly deformed mandibles diverge not only in the numbers of teeth, but in conformation and patterns of the spurs, bristles, and setae and in the general morphology of the mandible. The number of subapical teeth greatly increase from four (Fig. 8b) to 11 and 16 (Fig. 8a and 8e, respectively). Similarly, the number of apicodorsal teeth can increase from two (Fig. 8a, 8b) to four, five, six, eight, and nine (Fig. 8i, 8c, 8g, 8d, and 8h, respectively). The pecten on the mandibles in Fig. 8e, 8c, 8d, 8g, and 8i are irregularly patterned and misshapen as are the apicodorsal spurs. The mandible in Fig. 8a has two complete parallel rows of mandibular pecten and the distal end of the mandible is elongate and misshapen. The apices of the mandibles in Fig. 8g, 8h, and 8i are greatly elongate whereas those in Fig. 8f, 8k, and 8j are strongly reduced. The mandible in Fig. 8l is blunted, heavily fused, and the teeth greatly reduced and overlapping.

4. Normal and deformed premandibles

Premandibles are movable, inwardly curved appendages (Fig. 9a) located on either side of the ventral surface of the labrum attached to the tormal bar. Each has two apical teeth, or rami, identified herein as *a* and *b* for purposes of discussion, and a brush of small microtrichia, the premandibular brush, on the mesal margin (Saether 1980). The premandible probably functions in food manipulation.

Premandible deformities involve the number of apical teeth, the configuration of the premandible as a unit, and/or the configuration of individual teeth. Premandibles with a single tooth (Fig. 9e) probably are the most common form of deformity in the premandible, but examples with up to five teeth (Fig. 9r, 9s) have been found. The progression toward the single tooth configuration can proceed either through the loss of the *a* or *b* tooth or the fusion of both teeth. The remnant of the slender *a* tooth in Fig. 9b is gradually lost in Fig. 9c and 9d to the point where distinguishing between double- and single-toothed premandibles becomes problematic. Alternatively, in Fig. 9i–9l, the inner *b* teeth are much shorter than normal. Both teeth on the premandibles in Fig. 9f and 9g are shorter than normal. This progression seems to proceed toward the single-tooth configuration in Fig. 9h or the loss of both teeth in Fig. 9m. In the latter example, only remnants of the two teeth remain and the premandible is strongly atrophied.

The configuration and number of teeth can also vary. The premandibles in Fig. 9n and 9o each have three teeth, but in the latter, the third tooth is superimposed on the *b* tooth rather than being in line with the *a* and *b* teeth as in Fig. 9n. The premandible in Fig. 9l has a long dark structure projecting distally from the basal area of the teeth. This may represent a third tooth, but this seems doubtful. The premandibles in Fig. 9p and 9q have four teeth whereas those in Fig. 9r and 9s have five. In the latter example (Fig. 9s), the teeth are badly misshapen and atrophied.

5. Normal and deformed epipharyngeal pecten

The epipharyngeal pecten is a shallow U-shaped, transverse, toothed bar (Fig. 10a) located directly behind the labral margin between the tormal bar and ungula. The strongly shaped teeth generally increase in size from the outer margins inwards towards the center. Its function is unknown although it probably is involved in food manipulation.

Deformities in the epipharyngeal pecten range from fusing of the teeth to overlapping, asymmetry, stunting, and disjunction of the epipharyngeal structure. Fusion can begin with the junction of two central (Fig. 10b, 10c) or lateral (Fig. 10h, 10i) teeth and progress to the point where the central (Fig. 10g) and/or outer (Fig. 10m) teeth resemble scales rather than teeth. Overlapping can range from simple overlapping of individual teeth (Fig. 10n) to more extensive overlapping (Fig. 10p, 10q).

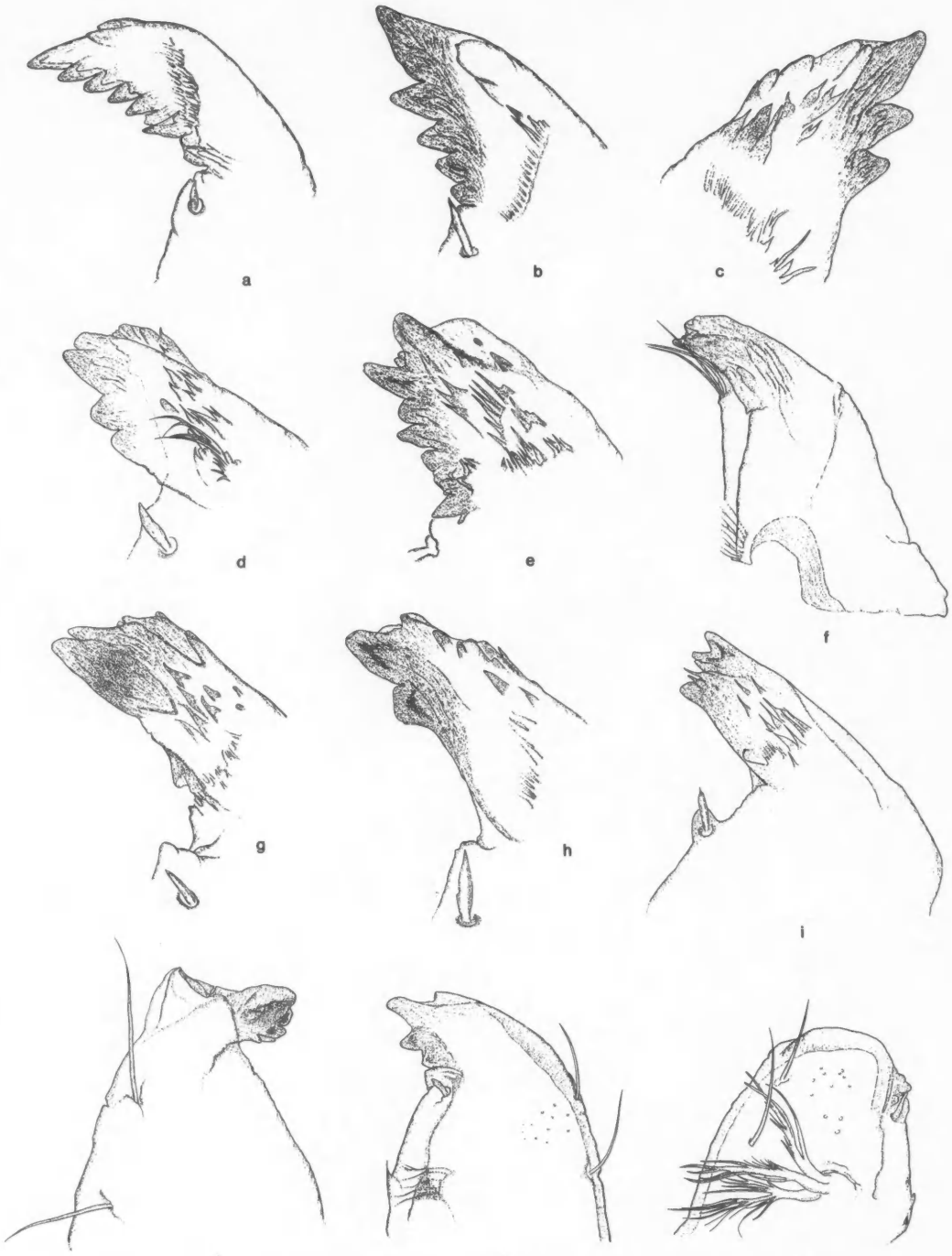


FIG. 8. Deformities in the mandibles of *Chironomus plumosus* type larvae: strongly deformed mandibles.

The transverse bar of the pecten can become progressively disjointed into two (Fig. 10r, 10s), three (Fig. 10t, 10u), and even four (Fig. 10v) individual sections which may overlap one another or be completely separated. In Fig. 10w, the transverse bar is separated into three distinct, coarsely toothed units. It is interesting to note that this arrangement and the broadly fused scale-like teeth in Fig. 10g and 10m suggest a trend towards the evolutionarily more primitive arrangement of three median

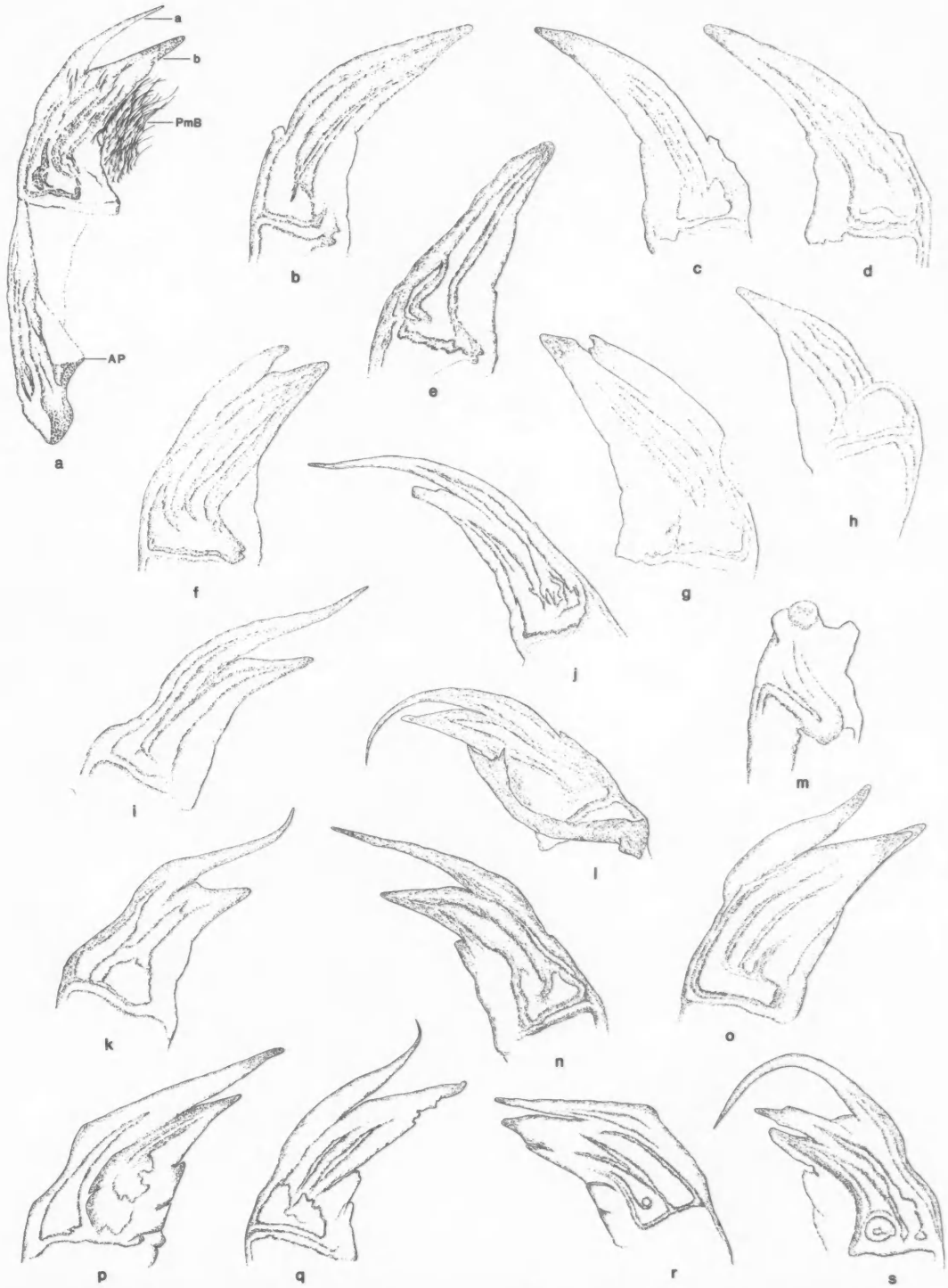


FIG. 9. Deformities in the premandibles of *Chironomus plumosus* type larvae: (a) normal premandible; (b–s) deformed premandibles. AP, articulating process; PmB, premandibular brush.

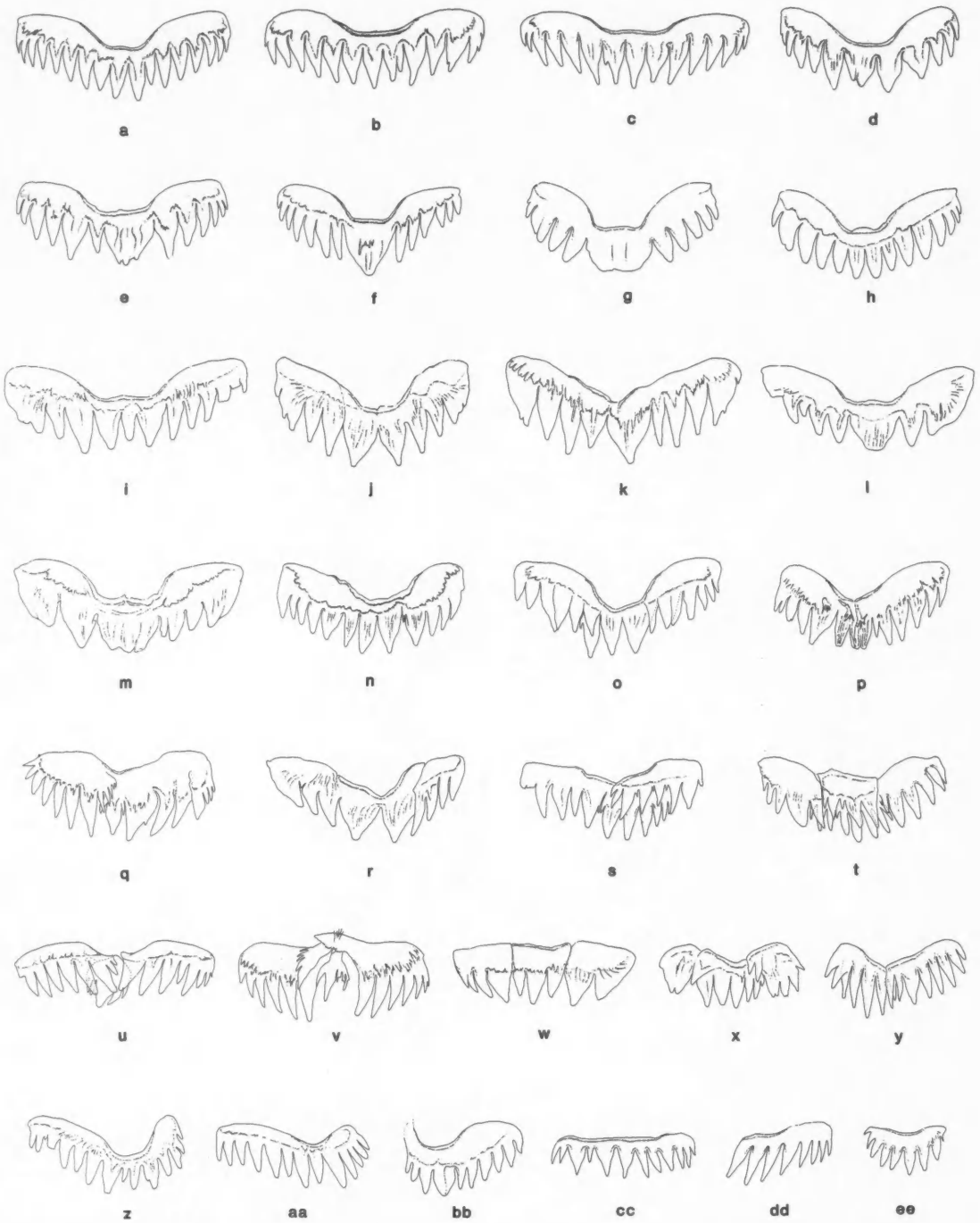


FIG. 10. Deformities in the epipharyngeal pecten of *Chironomus plumosus* type larvae: (a) normal epipharyngeal pecten; (b–ee) deformed epipharyngeal pecten.

scales in the epipharyngeal pecten (Saether 1980). (It is interesting to speculate whether or not contaminants promote reversion back to the more primitive condition.) The development of the pecten in Fig. 10z, 10aa, and 10bb is asymmetrical whereas in Fig. 10cc, 10dd, and 10ee, the pecten appear atrophied, withered, and undersized.

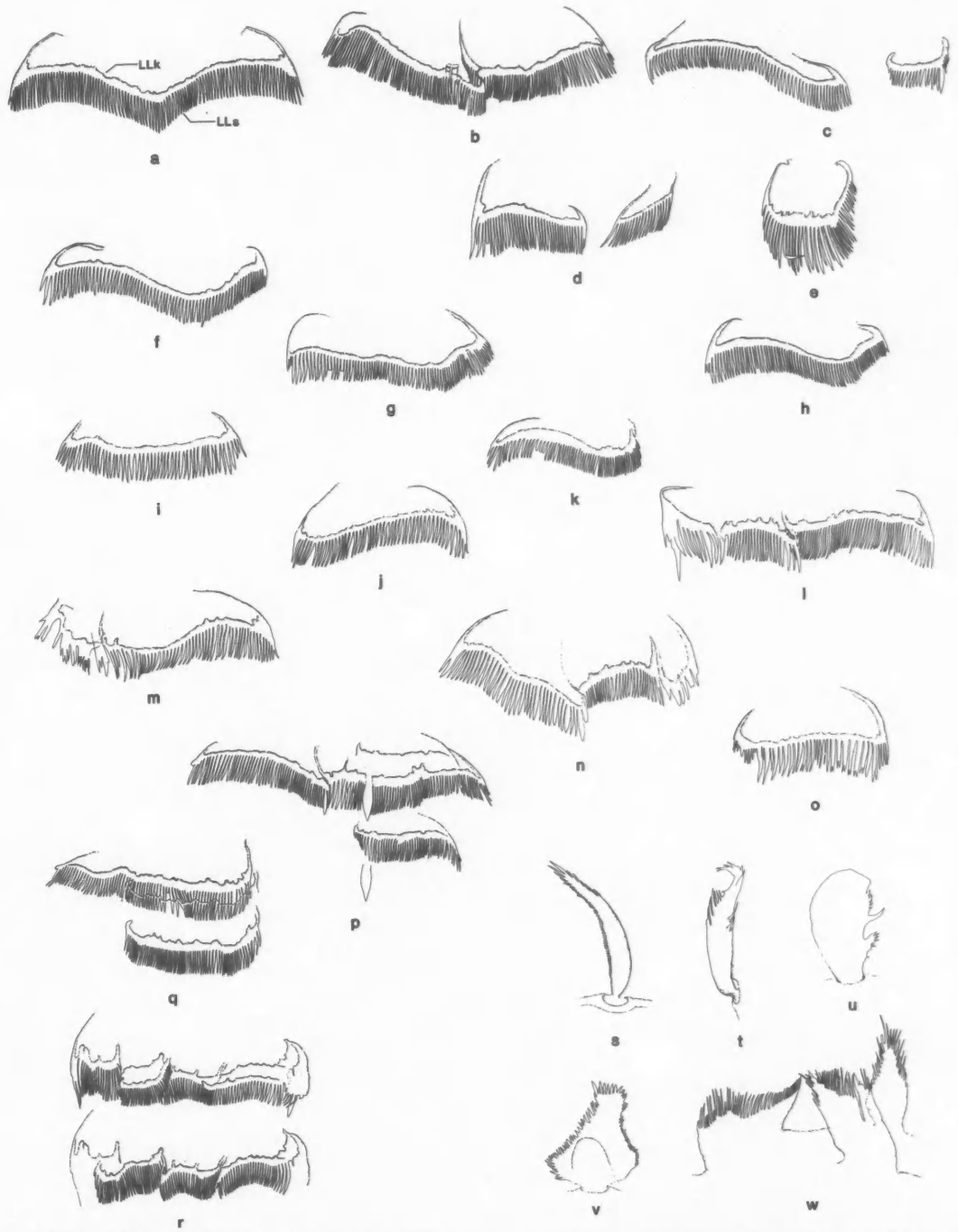


FIG. 11. Deformities in the labral lamellae and labral setae of *Chironomus plumosus* type larvae: (a) normal labral lamella; (b–r) deformed labral lamellae; (s) normal labral seta; (t–w) deformed labral setae. LLk, knuckle of labral lamella; LLs, spines of labral lamellae.

6. *Normal and deformed labral lamellae*

The labral lamella is a single, shallow V-shaped pectinate structure (Fig. 11a) located between the SI labral setae and the tormal bar (Saether 1980). Except at the extreme corners, the long, slender spines fringing the thickened knuckle of the lamella are generally uniform in length. Its function is unknown although it may possibly be sensory.

Deformities in the labral lamellae involve division along the

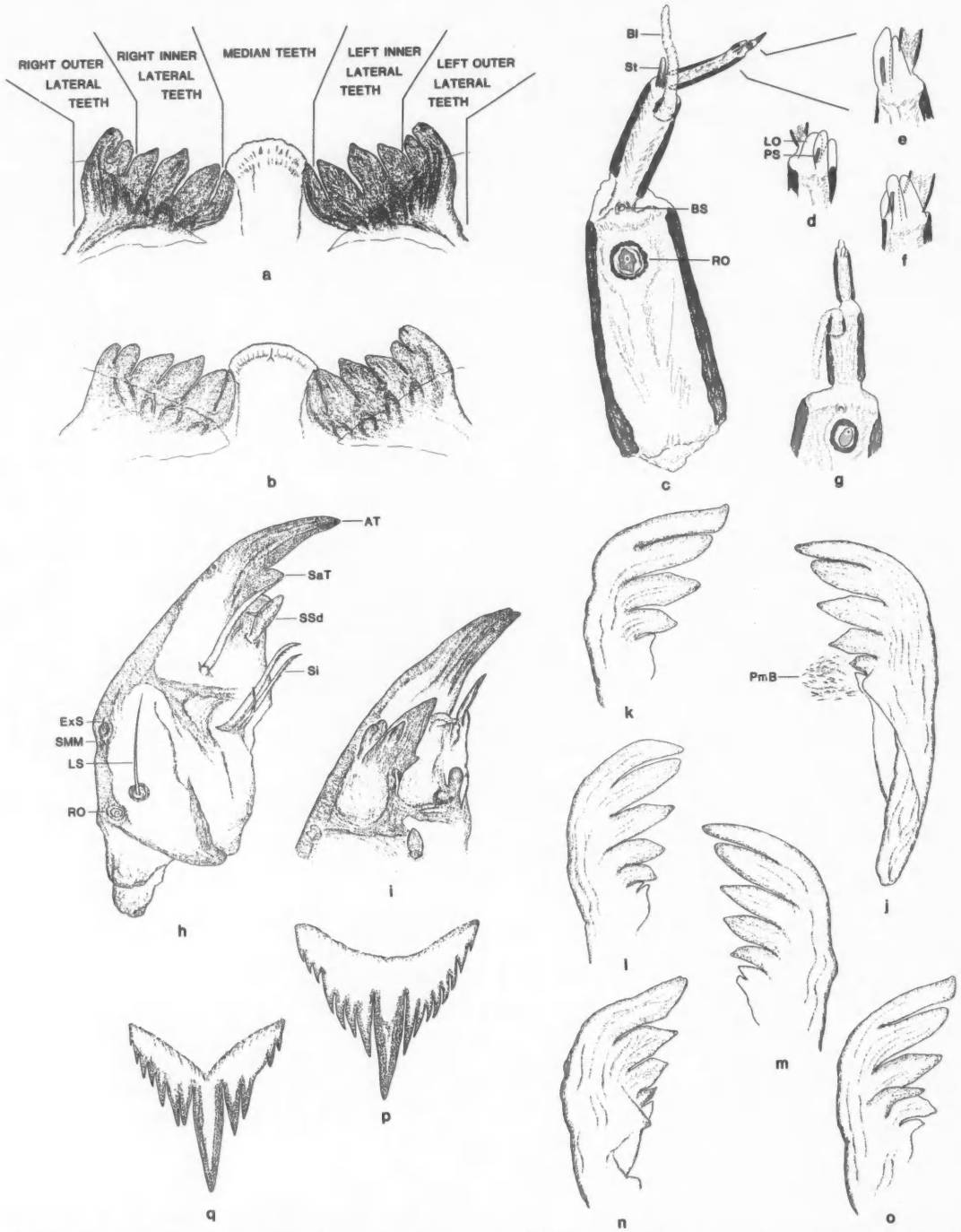


FIG. 12. Deformities in *Cryptochironomus* spp. larvae: (a) normal mentum; (b) deformed mentum; (c) normal antenna (with segment enlargements d–f); (g) deformed antenna; (h) normal mandible; (i) deformed mandible; (j) normal premandible; (k–o) deformed premandibles; (p) normal epipharyngeal pecten; (q) deformed epipharyngeal pecten. Bl, blade; St, style; PS, peg sensillum; LO, organs of Lauterborn; BS, basiconic sensillum; RO, ring organ; AT, apical tooth; SaT, subapical teeth; SSd, seta subdentalis; Si, setae interna; ExS, external seta; SMM, sensillum minusculum; LS, lateral seta; PmB, premandibular brush.

median suture line, reduction in size (in whole or in part), malformation of individual spines, and/or duplication and overlapping of individual sets of spines. Division of the lamella into two halves or "sets" is only partially complete in Fig. 11b, but in Fig. 11c and 11d, separation is complete. In Fig. 11c, the left half or "set" is much reduced in size compared with its counterpart, but in Fig. 11d, both are reduced. The lamella in Fig. 11e is symmetrical and bears both support arms, but it is strongly U-shaped and reduced in size. In contrast, the progressively smaller lamellae in Fig. 11f–11k are asymmetrical and appear to represent the development of only one half or "set" of the lamella.

The labral lamellae in Fig. 11l, 11m, and 11n appear incompletely divided into two or three sets of spines. In each example, irregular and abnormally shaped spines are present. In Fig. 11o, the lamella is symmetrical, but reduced in size and many of the spines are bifid.

The lamellae in Fig. 11p, and 11r are composed of numerous sets (two, three, and seven, respectively) of spines overlying one another. The sets are drawn separately to avoid confusion, with only the knuckle of the overlying sets outlined to show their superior position.

7. Normal and deformed labral setae

The anterodorsal part of the labrum bears four pairs of setae. Only the first pair, the SI, are noted here. The SI in *C. plumosus* type larvae are plumose with branches extending only partially down the outer side of the seta (Fig. 11s). Saether (1980) referred to the SI's as sensilla chaetica which suggests a mechanoreceptor role.

Deformities in SI labral setae vary widely from the mildly deformed example in Fig. 11t to the bizarre manifestation shown in Fig. 11w. Unless the specimen is carefully mounted, these types of deformities are difficult to detect.

Description of Morphological Deformities in *Cryptochironomus* Larvae

1. Normal and deformed antennae

The five-segmented antennae of *Cryptochironomus* differ (Fig. 12c) somewhat from that of *Chironomus* in that most of the sensillae which serve in mechano- and chemoreceptor roles are advanced distally by one segment. The organs of Lauterborn and peg sensilla are located between the third and fourth antennal segments. The peg sensilla is a long, post-like structure which frequently is difficult to see. There appears to be only one and this is shielded by two (enlargement Fig. 12e) or three (enlargement Fig. 12d, 12f) scale-like organs of Lauterborn. The heavier shielding around this fragile sensory organ possibly reflects an adaptation for the predatory lifestyle of *Cryptochironomus* larvae. The style is located between the third and fourth segments, while the blade is located subapically on the second segment. There appears to be no conjoined accessory blade as in *Chironomus*. A basiconic sensillum and ring organ are present on the basal segment. The single deformed antenna in Fig. 12g lacks distal segments IV and V and the style.

2. Normal and deformed menta

The mentum (Fig. 12a) has 15 teeth. The large central tooth is pale in colour and is flanked by smaller, closely conjoined dark teeth on either side. There are three larger inner lateral teeth on each side and three smaller outer lateral teeth, the outer two of which frequently appear only partially split. The dark concave outer teeth are also directed inwardly towards the large cutting edge of the central tooth, possibly another adaptation for its predatory role. The deformed mentum in Fig. 12b has two rather than three outer lateral teeth on the right side of the mentum.

3. Normal and deformed mandibles

The mandible of *Cryptochironomus* (Fig. 12h) has a single heavily sclerotized apical tooth flanked by two smaller dark subapical teeth. The seta subdentalis is spatulate and strong, while the mandibular brush is represented by a single heavy setae (Curry 1958). The setae internae are strong and barbed. A ring organ, lateral and external setae, and setae minusculum occupy the ventrolateral quadrant of the mandible.

In the badly deformed mandible in Fig. 12i, the two subapical teeth of the normal mandible have been replaced by four or five laterally located, misshapen teeth. Small blunted appendages also appear where the setae internae (Si) and mandibular brush should be located.

4. Normal and deformed premandibles

The premandibles of normal fourth instar larvae of *Cryptrochironomus* are equipped with six teeth and a premandibular brush along the inner margin (Fig. 12j). Deformed premandibles with five (Fig. 12k) and seven (Fig. 12l, 12m) teeth were frequently observed. Examples in which one of the six teeth was superimposed (Fig. 12n, 12o) appeared less frequently.

5. Normal and deformed epipharyngeal pecten

The epipharyngeal pecten is a single, triangular-shaped scale margined along two sides by small, sharp teeth terminating in one strong apical tooth (Fig. 12p). The deformed epipharyngeal pecten in Fig. 12q lacks a number of small teeth laterally and is deeply indented along the back margin.

Description of Morphological Deformities in *Procladius* Larvae

1. Normal and deformed antennae

The antenna of *Procladius* has four segments (Fig. 13n), the basal segment of which is much longer than the apical segments (enlargement Fig. 13o). The ring organ is located roughly 0.75× from the base of the basal segment. No basiconic sensillum appears to be present.

The antennal blade and conjoined accessory blade are located on the apex of the basal segment (enlargement Fig. 13p). Also located in this position are two tiny, dark-coloured, crescent-shaped structures similar to the structures located between the third and fourth segments on *Chironomus* antennae. Warwick (1985) suggested that these structures were peg sensilla. From their shape, these may be the same structures on *Procladius* antennae.

There are three structures located on the apex of the second segment (Fig. 13p). The structure of the larger of the three is very similar to the style on *Chironomus* antennae and probably serves the same function. The smaller two (referred to as sensory pegs SP_1 and SP_2 in Roback 1980) appear tube-shaped and heavy-walled. Each is mounted independently on individual pedestals rather than on the articulating membrane linking segments two and three. This would seem to rule out a mechanoreceptor role similar to that proposed for the organs of Lauterborn in *Chironomus* (Warwick 1985). Their structure more likely suggests a chemosensory role. According to Slifer (1970), thick-walled receptors of this type serve as contact chemoreceptors sensitive to stronger chemical stimuli. This interpreta-

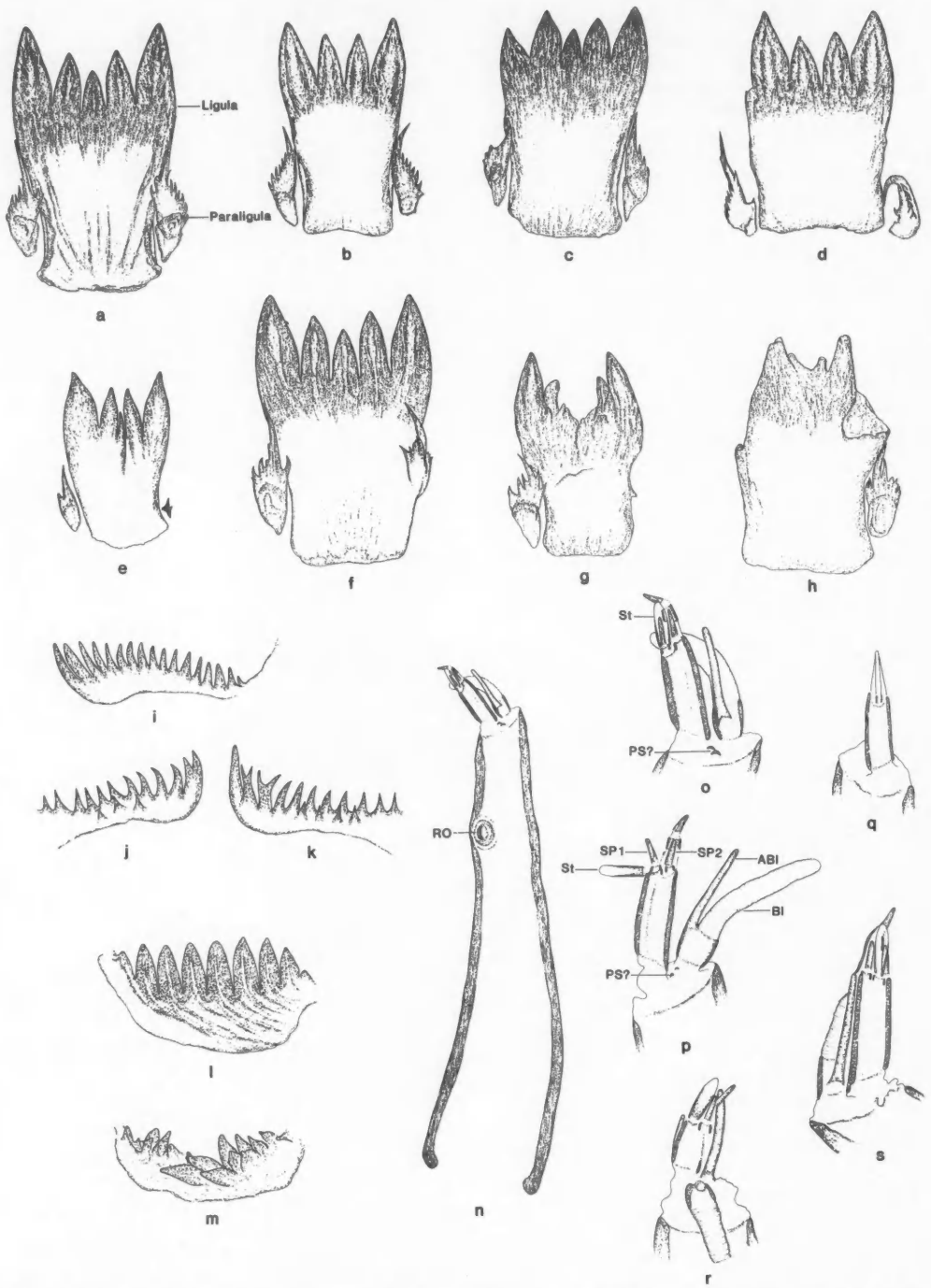


FIG. 13. Deformities in *Procladius* larvae: (a) normal ligula; (b–h) deformed ligula; (i) normal hypopharyngeal pecten; (j–k) deformed hypopharyngeal pecten; (l) normal hypopharyngeal comb; (m) deformed hypopharyngeal comb; (n) normal antenna (with segment enlargements o–p); (q–s) deformed antennae. Bl, blade; ABl, accessory blade; St, style; PS, peg sensillum; RO, ring organ; SP, sensory pegs.

tion makes sense in view of the predatory life style of *Procladius*.

Antennal deformities in *Procladius* take much the same form as in *Chironomus*. In Fig. 13q, the apical segments have been replaced by one (or two?) transparent, wedge-shaped structures which lie behind the one remaining pedestal-mounted sensory

TABLE 2. Frequency (%) of types of deformities in *Chironomus* spp. larvae from Tobin Lake Sites 2 and 17.

	Site 2		Site 17	
	No.	%	No.	%
Number of larvae with deformed				
menta	103	21.1	23	14.9
mandibles	40	8.2	7	4.6
premandibles	23	4.7	3	1.9
antennae	22	4.5	19	12.3
epipharyngeal pecten	57	11.7	11	7.1
labral lamellae	22	4.5	2	1.3
labral setae	5	1.0	0	0.0

TABLE 3. Frequency (%) of single and multiple deformities in *Chironomus* spp. larvae from Tobin Lake Sites 2 and 17.

	Site 2		Site 17	
	No.	%	No.	%
Number of larvae with				
single deformities	145	29.8	35	22.7
double deformities	45	9.2	12	7.8
triple deformities	8	1.6	2	1.3
quadruple deformities	3	0.6	0	0.0
Total	201	41.3	49	31.8

TABLE 4. Severity (ISAD) of antennal deformities in *Chironomus* spp. larvae from Tobin Lake Sites 2 and 17.

	Site 2	Site 17
Number of *Chironomus* spp. larvae	487	154
Number of larvae with deformed antennae	22	19
Frequency of deformed antennae (%)	4.5	12.3
Number of evaluated larvae	21	19
Total IMR	264	205
Average IMR	12.6	10.8
ISAD	0.54 ± 0.25	1.33 ± 0.76

tube. The apical segments appear to be missing on the example in Fig. 13r, but more importantly, the wall of the second segment is incompletely sclerotized and a structure, possibly the accessory blade, is mounted sublaterally on the wall of the second segment. In Fig. 13s, the walls of the second, third, and fourth segments are partially fused.

2. Normal and deformed ligula and paraligua

The ligula of the Tanypodinae is a flexibly mounted stabbing mouthpart which replaces the mentum in other chironomid subfamilies. In normal *Procladius*, the ligula characteristically has five dark brown to black points arranged in a concave row (Fig. 13a). The paraligula are located at the base of the ligula and have a variable number of teeth, mainly along the outer margin.

Ligula with four teeth (Fig. 13b) probably represent the most common form of abnormal ligula. This configuration may proceed through the progressive loss of an outer tooth as suggested in Fig. 13c and 13d. The paraligula can also be reduced (Fig. 13e) or become fused to the ligula (Fig. 13f). The ligula in Fig. 13g is strongly deformed and has seven additional teeth for a total complement of 12 teeth. The massively deformed ligula in Fig. 13h is fused and the teeth blunted. The "tooth" midway down the right side of the ligula probably represents the fused remnant of the right-hand paraligula.

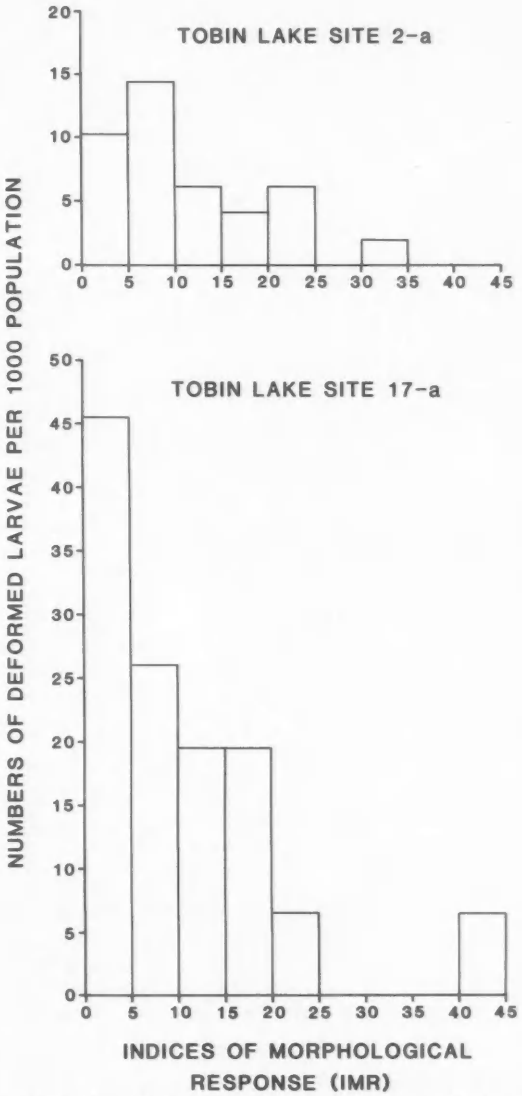


FIG. 14. Indices of Morphological Response in antennae by class for Tobin Lake Sites 2 and 17.

3. Normal and deformed hypopharyngeal pecten

The hypopharyngeal pecten are a transverse row of (generally) 8–16 simple teeth or spines (Fig. 13i) mediobasal on the hypopharynx (Saether 1980). The deformed examples in Fig. 13j and 13k have bifid teeth in their complement.

4. Normal and deformed hypopharyngeal combs

The hypopharyngeal combs are a set of five to nine triangular-shaped teeth (Fig. 13l; dorsomental teeth sensu Roback 1980) on the prementohypopharyngeal complex. The comb in Fig. 13m has 17 teeth, many of which are superimposed over one another and irregular in size.

TABLE 5. Frequency (%) of types of deformities in *Cryptochironomus* sp. larvae from Tobin Lake Sites 2 and 17.

	Site 2		Site 17	
	No.	%	No.	%
Number of larvae with deformed				
menta	2	1.6	1	2.2
mandibles	1	0.8	0	0.0
premandibles	4	3.3	1	2.2
antennae	1	0.8	0	0.0
epipharyngeal pecten	0	0.0	1	2.2

TABLE 6. Frequency (%) of types of deformities in *Procladius* spp. larvae from Tobin Lake Sites 2 and 17.

	Site 2		Site 17	
	No.	%	No.	%
Number of larvae with deformed				
ligula	7	2.0	0	0.0
ligula/paraligula	2	0.6	1	1.0
antennae	3	0.9	0	0.0
hypopharyngeal pecten	4	1.2	1	1.0
hypopharyngeal combs	1	0.3	0	0.0

Occurrence of Characteristic Deformities

Structural Deformities in *Chironomus* spp. Larvae

The frequency of deformities in *Chironomus* spp. larvae was significantly higher at Site 2 (41.3%) than at Site 17 (31.8%). Chi-square analysis showed that the proportion of deformed to nondeformed larvae was significantly different at the two sites ($\chi^2 = 4.39$ where $\chi^2_{0.05,1} = 3.84$). The difference in the frequency of deformities probably reflects the difference in contaminant burdens impinging on the two reservoir sites.

The frequencies of deformities in different morphological structures were generally higher at Site 2 than at Site 17 (Table 2). The only exception was the antennae in which case the frequency of deformities was significantly greater at Site 17. Except for the antennae, the significance of the differences in the proportions of deformities in these other structures was generally less than 95%. Chi-square values for menta (2.86) and labral lamellae (3.37) were significant between 90 and 95% (where $\chi^2_{0.10,1} = 2.71$) and chi-square values for epipharyngeal pecten (2.57), mandibles (2.32), and premandibles (2.32) were significant between 85 and 90% (where $\chi^2_{0.15,1} = 2.18$). Although less easily interpreted, these results are surprisingly good for simple numerical data which include no estimates of severity of response.

The deformities in individual larvae were also separated according to single and multiple deformities (Table 3) and tested by chi-square. The difference in the proportions of single deformities was significant at about 95% ($\chi^2 = 3.65$ where $\sigma^2_{0.05,1} = 3.84$). None of the multiple combinations differed significantly.

Antennal Deformities

The only numerical data to show clear statistical differences were in the antennae (Table 2). According to chi-square, the proportions of deformed and nondeformed antennae in the two fauna were significantly different ($\chi^2 = 11.95$ where $\chi^2_{0.001,1} = 10.83$).

Antennal deformities were about 3 times more frequent at Site 17 than at Site 2, but the severity of deformities was greater at Site 2 (Table 4). According to Warwick (1985), the Index of Morphological Response (IMR) provides a measure of the severity of deformation in individual antennae. The distribution of IMR values in Fig. 14 shows that the greater proportion of deformities from Site 2 occurred in the higher response ranges, that is, in more severe IMR classes 6–10, whereas those from Site 17 occurred in less severe IMR classes 0–5. The difference in severity is further reflected in the average values for IMR (Table 4). The higher value at Site 2 agrees with the contention that the contaminant burden is greater at this site than at Site 17.

The distribution of individual IMR rankings from Sites 2 and 17 was compared using Cochran's modified *t*-test for dissimilar variances (Dixon and Massey 1969). The test indicated that within individual rankings the differences in severity were significant at about 95% ($t = 1.92$ where $t_{0.05,192} = 1.96$). For a single variable, this is quite good. It will be interesting to note as the various indices needed for an Index of Total Morphological Response (TMR) become available whether their inclusion will significantly improve these results and the ability of the test to accurately define differences between sites as Warwick (1985) suggested.

The values for the Index of Severity of Antennal Deformation (ISAD), a method developed by Warwick (1985) to assess the severity of deformities in larval antennae, were 0.54 and 1.33, respectively, for Site 2 and 17 (Table 4). At face value, these results appear to run contrary to the contention that Site 2 is the more seriously contaminated of the two sites. However, when interpreted in terms of quantal dose–response theory, as discussed below, the data do support the contention that Site 2 is the more seriously contaminated site.

Structural Deformities in *Cryptochironomus* spp. and *Procladius* spp. Larvae

The incidence of deformities in the different morphological structures of *Cryptochironomus* sp. larvae differed only marginally between Sites 2 and 17 (Table 5). In terms of severity, the only seriously deformed larva came from Site 2. The multiple deformities in this larva included a deformed mandible (Fig. 12i), a deformed antenna (Fig. 12g), and a deformed premandible (Fig. 12n). With this single exception, the deformities in the other larvae from both sites were single and relatively mild.

The incidence of deformities in the different morphological structures of *Procladius* spp. larvae also differed only marginally between Sites 2 and 17 (Table 6). In terms of severity, the more seriously deformed larvae came from Site 2. Although the severity of these has yet to be quantified, their distribution tends to support the contention that Site 2 is the most seriously contaminated of the two Tobin Lake sites.

Discussion

Few reports in the literature dealing with deformities in chironomid larvae describe these in detail. Klink (1984) presented an alphanumeric system for categorizing deformities in the teeth of the larval mentum. This is basically a cataloguing system and does not attempt to evaluate severity of deformation. Warwick (1985) formulated an indexing scheme for quantifying the severity of deformities in the antennae of the genus *Chironomus*. The index was left open-ended so that it (1) could be

adapted to the antennae of other species and (2) would eventually form part of a greater index including estimates of the severity of deformities in other structures of the larval head and body in an Index of Total Morphological Response.

The frequencies of deformities reported here for *Chironomus* spp. larvae from Sites 2 and 17 (41.3 and 31.8%, respectively) are high compared with values previously published in the literature. Two reasons may account for this: (1) the deformities listed here include many new structures for which deformities have never before been described and (2) the definition of deformities currently employed may include some individuals expressing normal rather than abnormal variation.

According to the definition presented in Warwick (1988), a morphological structure is considered deformed if its configuration departs from the normal. This definition is necessarily broad and all-inclusive because the boundaries between normal and abnormal variation have yet to be established for most morphological structures. The transition from normal to abnormal variation represents a continuum of response, and establishing the "limits of detection" for each structure may be important. Until suitable boundaries are established, however, some questionable examples probably will be included in estimates of frequency using the current definition.

As a case in point, the listings for deformed epipharyngeal pecten include a number of specimens displaying only very mild fusing between adjacent teeth (Fig. 10b, 10c). The question is whether these specimens are deformed or do they simply represent variants of normal structure. At the present time there is no satisfactory way to determine whether these are indeed true deformities. However, the problem may be alleviated in large measure when a satisfactory method for evaluating the severity of deformities in these structures can be established. It may be that epiphryngeal pecten are not good indicators of contaminant stress, but I suspect this is a case where simple frequency analysis is not adequate for evaluating stress response.

The importance of estimating severity of deformation is an integral part of estimating individual responses to contaminant stress. According to the National Research Council of Canada (1985), "percent response may show changes over time and space where mean response may not, (but) condensing data to this extent could cause loss of very important (response) information " (author's parentheses). Two sites with similar frequencies of deformities may be thought to be equally exposed to contaminants, but in actual fact may differ widely in the degree to which they are exposed. Without some means of dealing with the range or severity of response, the difference may go undetected and the real situation incorrectly assessed (National Research Council of Canada 1985).

One technique that is available for evaluating severity of response is ISAD. At first glance, the results for ISAD in this study seem to argue against the contention that Site 2 is the more heavily contaminated of the two Tobin Lake sites. The ISAD value for Site 2 (0.54) is lower than that for Site 17 (1.33), the less highly contaminated site. However, because n in the calculation of ISAD = $\Sigma IMR/n$ is based on the total number of specimens examined (i.e. the total larval population) rather than just the number of larvae with deformed antennae, the data must be interpreted in terms of quantal dose–response (Chan et al. 1984). It then becomes important to determine which side of the bell-shaped distribution curve each datum falls. If the datum for Site 2 is placed on the right-hand side of the distribution curve rather than on the left, the results then become consistent with the pattern shown by the frequency of deformities (41.3%) and the average value for IMR (12.6), both of which support the contention that Site 2 is the more seriously contaminated site, as common sense would dictate.

Warwick (1985) noted the same curiously inverted pattern in earlier work on Tobin Lake. In that study, lower ISAD values were found in areas of the reservoir where one would anticipate higher levels of contamination based on considerations of hydraulic energy and basin mechanics. Site VI, located in the deeper water area of the reservoir, might be expected to be more highly contaminated than Site V located between Site VI and the inflow area of the Saskatchewan River (Fig. 1). However, the ISAD value for Site VI (1.19) was lower than that (1.64) for Site V. As in the present study, the apparent contradiction can also be explained by quantal dose–response theory. If the two data points are positioned on the right-hand side of the quantal dose–response curve, Site VI then becomes the more contaminated of the two sites.

The placement of the datum from Site 2 on the right-hand side of the curve also explains in part the apparent inversion in the frequency of deformities in the antennae. Warwick (1985) noted that as the concentration of contaminants increased, response shifted from the antennae to other morphological structures. Placement of the ISAD datum on the right-hand side of the curve implies that the sensitivity of antennae is declining and response has begun to shift to the harder structures. This not only explains why the frequency of antennal deformities is lower at Site 2 (4.5%) than at Site 17 (12.3%), but also why the cumulative frequency of deformities is greater at Site 2 (41.3%) than at Site 17 (31.8%).

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Time Series Models of the Maine Lobster Fishery: The Effect of Temperature

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I used Box–Jenkins transfer function models to analyze the relationship between water temperature and Maine lobster catch and catch-per-unit-effort (CPUE). I first modelled catch and CPUE with univariate autoregressive – integrated moving average (ARIMA) models to provide a basis for comparison with transfer function models. Time series models were constructed for annual Maine lobster landings during two periods: 1928–85 and 1945–85; catches during the latter period were assumed to be less dependent on changes in fishing effort. I also modelled annual CPUE for the period 1930–85 and monthly landings for 1968–85. Landings and CPUE for 1986 were held in reserve to check forecast estimates. An immediate temperature effect (lag 0–1 yr) was demonstrated for each annual series. This result is consistent with known aspects of lobster biology; activity levels and hence vulnerability to capture increase with water temperature. In addition, the probability of molting increases with increasing water temperature, affecting the short-term supply of legal-sized lobsters. A significant effect of temperature at a 6-yr lag was also indicated, but only for the 1945–85 catch series. Delayed effects of this type may indicate environmental influences on natality or survival during early life history stages. Time series models for the Maine lobster fishery provided forecasts for 1986 catch and CPUE which differed by no more than 4% of the actual 1986 levels.

On utilise des modèles de fonction de transfert Box–Jenkins pour analyser la relation entre la température de l'eau, les prises de homard dans l'État du Maine et les prises par unité d'effort (PUE). En premier lieu, on a modélisé les prises et les PUE à l'aide de modèles ARIMA comme base de comparaison à des modèles de fonction de transfert. On a ensuite élaboré des modèles en série chronologique pour les débarquements annuels de homards du Maine au cours de deux périodes, soit 1928–1985 et 1945–1985; on a supposé que les prises au cours de cette dernière période étaient moins dépendantes de variations de l'effort de pêche. On a aussi modélisé les PUE annuelles pour la période 1930–1985 et les débarquements mensuels pour la période 1968–1985. Les débarquements et les PUE observés en 1986 ont été réservés pour vérifier les prévisions. On a observé une incidence immédiate de la température (décalage de 0 à 1 an) dans chaque série annuelle. Ceci concorde avec nos connaissances sur la biologie du homard : les niveaux d'activité et donc la capturabilité augmentent en fonction d'une augmentation de la température de l'eau. De plus, la probabilité d'une mue augmente en fonction d'une augmentation de la température de l'eau, ce qui influe sur les approvisionnements à court terme de homards de taille légale. On a aussi noté une incidence importante de la température qui a montré un décalage temporel de six ans mais seulement dans le cas de la série des prises notées de 1945 à 1985. Des incidences retardées de ce genre peuvent indiquer des influences environnementales sur la natalité ou la survie durant les premiers stades du cycle vital. Les modèles en série chronologique de la pêche du homard au Maine ont donné des prévisions des prises et des PUE pour 1986 qui ne s'écartaient pas plus de 4 % des niveaux réels observés en 1986.

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Variability in yield and productivity of harvested fish and invertebrate populations has frequently been attributed to environmental influences. Cushing (1982) summarized environmental effects on trends in abundance of marine fish and invertebrates. The potential role of water temperature, in particular, has been examined in considerable detail (Cushing 1982; Shepherd et al. 1984; Sissenwine 1984). Temperature can have direct effects on survival, growth, and distribution of marine organisms. Water temperature can also have indirect effects on production in higher trophic levels by influencing primary or secondary production. In other instances, temperature may simply be correlated with other variables that have direct effects on production (e.g. upwelling).

Possible relationships between temperature and yield of fish and invertebrate stocks in the Gulf of Maine have been extensively studied (Taylor et al. 1957; Sutcliffe et al. 1977), with particular emphasis on the American lobster, *Homarus americanus* (Dow 1964, 1969, 1976, 1977, 1978). Flowers and Saila (1972) developed multiple regression predictors for Maine lobster yield based on present and lagged temperature values. Orach-Meza and Saila (1978) constructed a polynomial distributed lag model for forecasting Maine lobster catches. Evidence

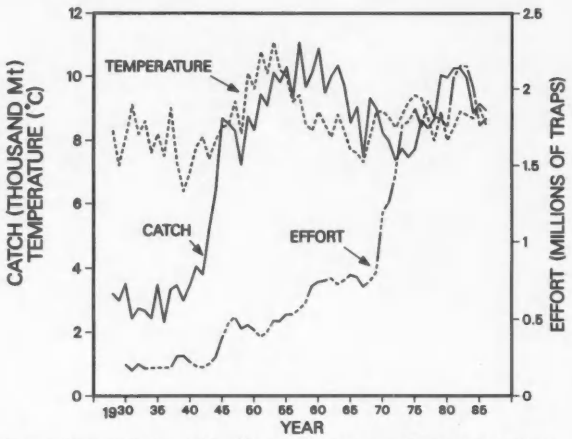

FIG. 1. Maine lobster yield, effort, and mean annual water temperature at Boothbay Harbor, Maine, for the period 1928–86.

for temperature-related effects on lobster yield in other regions is less clear. Harding et al. (1983) found no relationship between temperature and lobster landings along the Atlantic coast of Nova Scotia and in the Gulf of St. Lawrence. Marchesseault (1977) examined temperature–yield relationships for the Massachusetts lobster fishery; no delayed temperature effect was found, although evidence for an immediate effect was noted.

Here, I apply time series analysis to examine the relationship between water temperature and Maine lobster yield and catch-per-unit-effort (CPUE). Box and Jenkins (1976) described a flexible class of stochastic models for time series modelling and forecasting. Univariate time series models are constructed as linear functions of past values of the series and/or previous random shocks (or errors). The former represent autoregressive components, while the latter are moving average terms (Box and Jenkins 1976). Forecasts are generated under the assumption that the past history can be translated into predictions for the future. Box and Jenkins (1976) also described methods for objectively relating multiple time series. The individual series are first filtered (reduced to white noise) to remove the effects of autocorrelation within each series and then crosscorrelated to identify the appropriate model structure. This approach is effective in eliminating spurious correlations between the input (independent) and output (dependent) variables.

Applications of time series analysis to fishery-related problems have been described by Jensen (1976, 1985), Boudreault et al. (1977), Saila et al. (1980), Kirkley et al. (1982), Mendelssohn (1981), and Mendelssohn and Cury (1987). Transfer function models have also been described for natural populations by Hacker et al. (1975), Poole (1976), and Jenkins and Alavi (1981) and for a mesocosm system by Keller (1987). Applications of intervention analysis (Box and Tiao 1975) for modelling discrete impacts (or interventions) have been described by Noakes (1986), Madenjian et al. (1986), and Fogarty (1988). These studies document the predictive capabilities of Box–Jenkins models and, for multivariate models, their utility in empirically identifying structural relationships among variables.

Methods

Data Base

An uninterrupted series of annual catch records was available for the Maine lobster fishery for the period 1928–86 (Fig. 1; Dow 1976; Thomas et al. 1983; J. S. Krouse, Maine Department of Marine Resources, W. Boothbay Harbor, ME, pers. comm.), providing the basis for a long-term analysis of trends in coastal Maine lobster catches. Flowers and Saila (1972) suggested that high effort levels after about 1945 reduced the effective fishing power per trap. Accordingly, I considered the 1945–86 catch series separately because it appeared to be less dependent on changes in fishing effort. A monthly series of catch records from January 1968 to December 1986 (Thomas et al. 1983; J. S. Krouse, pers. comm.) was used to develop a seasonal model for the Maine lobster fishery.

The annual number of traps fished was available for 1930–86 (Fig. 1). I also developed time series models for the CPUE (kilograms per trap) series. Catch per effort is presumably a better index of population size than catch. Although the trap effort index is relatively crude, more detailed measures of effort are available only since 1968.

Water temperature has been monitored daily at the fishery research station at Boothbay Harbor, Maine, since 1905 (Churchill 1988; Fig. 1). Prior to October 1950, thermometer readings were used to record surface temperature several times daily; since then, continuous thermister readings have been taken at a depth of 5.5 m (Churchill 1988). Despite the potential bias introduced by changes in methodology, the Boothbay Harbor temperature series agrees well with other temperature records for this period in the northeastern United States (Sissenwine 1974; Skud 1982).

Analysis

Statistical procedures followed the iterative Box–Jenkins approach of model identification, estimation, and validation. The final phase, model interpretation, is particularly important (Jenkins 1979); the principal focus of this paper is interpretation of the relationship between temperature and yield.

Model identification is based on characteristics of the autocorrelation and partial autocorrelation functions of a stationary time series (Box and Jenkins 1976). The series must be stationary both in mean level and variance; transformation may be required to ensure homogeneity of variance. To check for violations of the assumption of variance stationarity, each series was first arbitrarily divided into segments and mean–range plots were examined (Jenkins 1979). In this paper, I consider models based on $\log_e$-transformed data because there was some indication of increased variability at high catch and CPUE levels and environmental effects are expected to be multiplicative rather than additive (Ricker 1975). Retransformation was made using the approximate bias correction suggested by Granger and Newbold (1977).

For series that are nonstationary in level, it may be necessary to difference the series (i.e. form the series $z_t = x_t - x_{t-n}$ where z_t is the differenced series, x_t is the original variable, and n is the period of differencing).[1] Once stationarity is assured, an appropriate model containing autoregressive and/or moving average terms may be specified. The general model form is

$$z_t = \phi_1 z_{t-1} + \phi_2 z_{t-2} \ldots + \phi_p z_{t-p} + a_t - \theta_1 a_{t-1} - \theta_2 a_{t-2} \ldots - \theta a_{t-q} + \Theta_0$$

[1]For notational convenience, it is possible to define the "backshift" operator, B^n, which indicates lagged variables ($B^n x_t = x_{t-n}$). The backshift operator may be used to indicate a differenced series, viz. $(1 - B^n)x_t = x_t - x_{t-n}$.

TABLE 1. Parameter estimate and associated statistics for a univariate autoregressive model for 1982–85 lobster yield. T is the t-ratio for the parameter, Q is the χ^2 statistic for autocorrelation in the residuals (through lag 24), and D is the Kolmogorov–Smirnov statistic for normality applied to the model residuals. Same information is supplied for a transfer function model. Signs of the coefficients follow standard Box–Jenkins notation with use of the backshift operator. *Significant at $p < 0.05$; **nonsignificant at $p > 0.05$.

Parameter	Lag	Estimate	SE	T	Q	D
			Univariate			
ϕ_1	1	−0.414	0.121	3.43*	21.93**	0.102**
			Transfer			
ω_0	0	0.552	0.223	2.45	19.34*	0.129**
ω_1	1	0.664	0.222	2.99		
ϕ_1	1	−0.318	0.130	−2.45		

where z_t is the time series variable (after differencing and/or transformation if necessary), a_t are independent normally distributed random shocks (error terms) with zero mean and constant variance, ϕ_i are autoregressive parameters and θ_i are moving average parameters subject to bounds of stationarity and invertibility, respectively (Box and Jenkins 1976), and Θ_0 is a deterministic trend parameter.

Univariate models were first fit for the two annual catch series (1928–85 and 1945–85), the 1930–85 CPUE series, and for the monthly catch series (1968–85) to provide a basis for comparison with transfer function models. Catch and CPUE data for 1986 were held in reserve to check forecasted values and were not used in estimation.

Analyses were performed on the "centered" series (i.e. the mean was subtracted from each observation). Following tentative model identification, maximum likelihood estimates of the parameters were made and the residuals of the model checked to ensure independence and normality. Independence of residuals was checked using the portmanteau test of Box and Jenkins (1976). Normality of residuals was examined using the Kolmogorov–Smirnov statistic (D) for large sample sizes ($N > 50$) and the Shapiro–Wilk statistic (W) for $N < 50$.

Development of transfer function models depends on model identification based on the crosscorrelation between the stationary dependent and independent variables. It is recommended that the series be "prewhitened" prior to analysis (Box and Jenkins 1976). In the bivariate case, a univariate model is developed for the input variable and then applied to both variables. The residuals of both series (white noise) are then crosscorrelated to determine the structural relationship between the variables. In this paper, a prewhitened series will be referred to as filtered.

The general form of a bivariate model is

$$z_t - \delta_1 z_{t-1} - \delta_2 z_{t-2} \cdots - \delta_r z_{t-m} = \omega_0 y_{t-b} - \omega_1 y_{t-b-1} \cdots \omega_n y_{t-b-n} + \eta_t$$

where z_t is the output (dependent) series, y_t is the input series, δ_i and ω_i are model parameters, and η_t is an error term which may be modelled as a univariate autoregressive – integrated moving average (ARIMA) process. The input and output variables are differenced and/or transformed as necessary. For a detailed overview, see Box and Jenkins (1976).

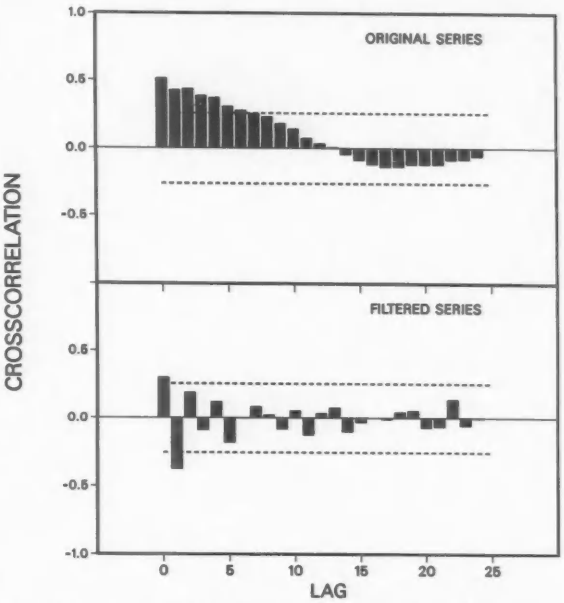

FIG. 2. Crosscorrelation between the 1928–85 yield and temperature for the original (upper) and filtered (lower) series. Values extending beyong the broken lines are significant at the 0.05 level.

Results

Annual Catch (1928–86)

A univariate model was first constructed for the catch series to provide a basis for comparison with transfer function models. The slow decay of the autocorrelation function of the original series clearly indicated nonstationarity; accordingly, first differences were taken. Examination of the autocorrelation and partial autocorrelation functions of the differenced series indicated that a first-order autoregressive model was appropriate. Parameter estimates for the final model are provided in Table 1. Attempts to "overfit" the model by inclusion of additional autoregressive and moving average terms indicated that a more detailed model was unnecessary. The final model was of the form

$$(1 - \phi_1 B)(1 - B)(\ln C_t) = a_t$$

where C_t is the catch series, ϕ is an autoregressive parameter, and B is the backshift operator. As a check on the adequacy of the model, the observed 1986 catch (8938 Mt) was compared with the forecast of 9339 Mt.

Development of a transfer function model incorporating temperature effects required filtering to remove within-series correlation. A second-order autoregressive model was developed for the $\log_e$-transformed temperature series. The final temperature model was

$$(1 - 0.491B - 0.234B^2)(\ln T_t - \mu) = a_t$$

where T_t is the temperature series and μ is the mean of transformed temperature series. The temperature model was applied to both series and the crosscorrelation between the residuals of both series was then examined. The crosscorrelation between the unfiltered catch and temperature series was

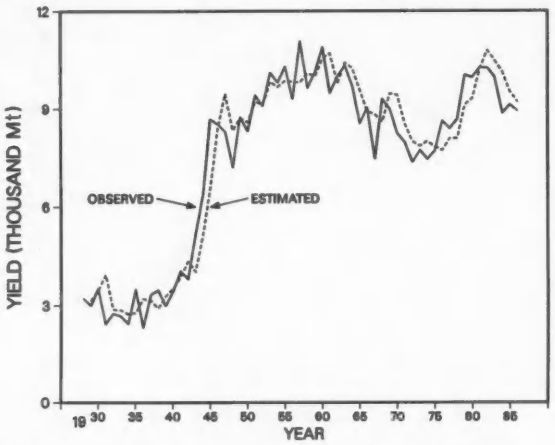

FIG. 3. Comparison of the observed (solid line) and estimated (broken line) catch series for the period 1928–85. Observed and predicted catches for 1986 are also provided.

TABLE 2. Parameter estimates and associated statistics for a univariate autoregressive model for 1945–85 lobster yield. T is the t-ratio for the parameter, Q is the χ^2 statistic for autocorrelation in the residuals (through lag 24), and W is the Shapiro–Wilk statistic for normality applied to the model residuals. Same information is supplied for a transfer function model. Signs of the coefficients follow standard Box–Jenkins notation with use of the backshift operator. *Significant at $p < 0.05$; **nonsignificant at $p > 0.05$.

Parameter	Lag	Estimate	SE	T	Q	D
			Univariate			
ϕ_1	1	0.406	0.148	2.74*	26.77**	0.975**
ϕ_2	2	0.344	0.158	2.32*		
			Transfer			
ω_0	0	0.521	0.202	2.57*	18.63**	0.987**
ω_6	6	−0.439	0.176	−2.49*		
ϕ_1	1	0.641	0.138	4.64*		

characterized by large positive correlations; however, the crosscorrelation between the filtered series showed significant correlations only at lags of 0 and 1 yr (Fig. 2). Development of a transfer function model (Table 1) resulted in a 10% reduction in residual variance relative to the corresponding univariate model. The significant temperature effect at lags of 0 and 1 yr implies that temperature influences catchability. The relationship between the observed and fitted catch series is illustrated in Fig. 3 for the transfer function model. The 1986 catch forecast using the transfer function model overestimated the actual yield by 3.2%; the predicted level was 9228 Mt.

Annual Catch (1945–86)

Development of univariate and transfer function models for the 1945–85 series followed the procedure outlined above; however, it was not necessary to difference the catch series. The pattern of autocorrelations and partial autocorrelations of the catch series indicated that a second-order autoregressive model was appropriate. Estimation and subsequent diagnostic checking of the model indicated that the residuals were independent, normally distributed random variables (Table 2). The 1986 forecast estimate of 9046 Mt for the univariate catch model overestimated the actual 1986 catch but provided a slightly better prediction than either the univariate or transfer function models based on the 1928–85 catch series.

A second-order autoregressive model was found to be appropriate for the 1945–85 temperature series. The final temperature model was

$$(1 - 0.475B - 0.269B^2)(\ln T_t - \mu) = a_t$$

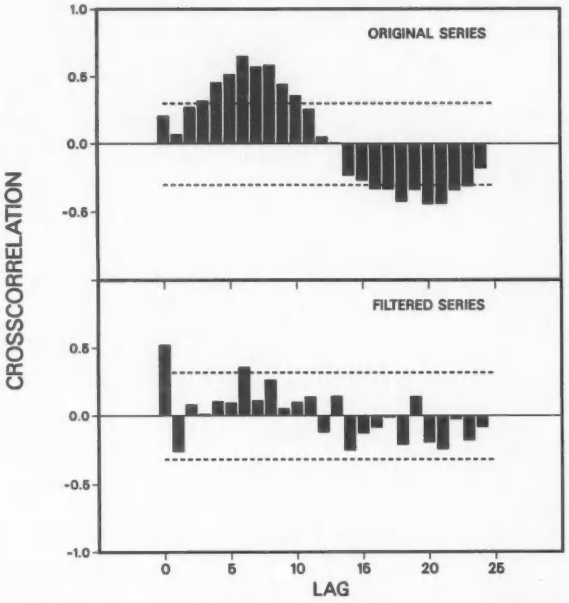

FIG. 4. Crosscorrelation between the 1945–85 lobster yield and temperature for the original (upper) and filtered (lower) series. Values extending beyond the broken lines are significant at the 0.05 level.

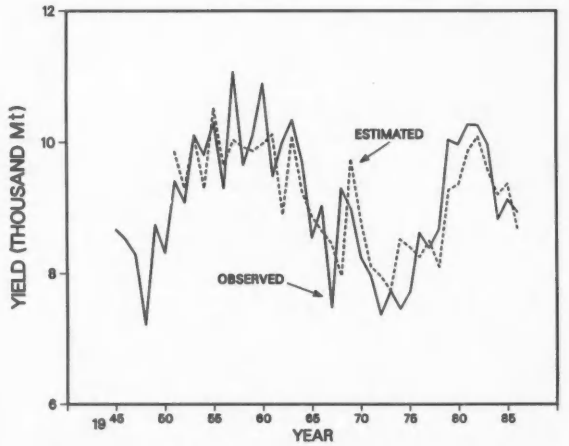

FIG. 5. Comparison of the observed (solid line) and estimated (broken line) catch series for the period 1945–85. Observed and predicted 1986 catch levels for 1986 are also provided.

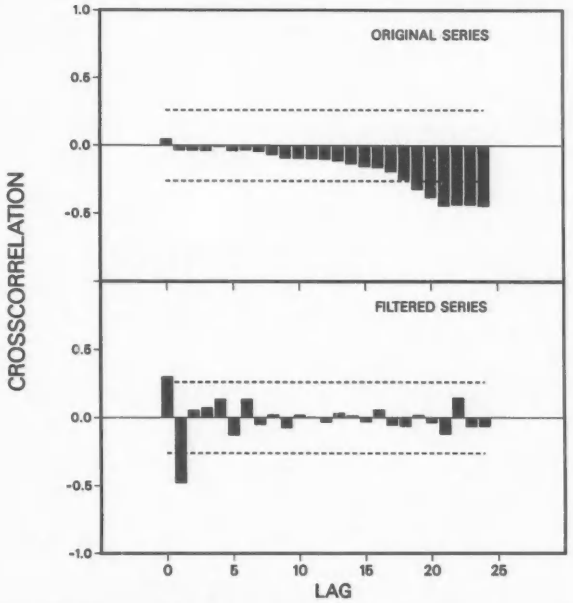

FIG. 6. Crosscorrelation between the 1930–85 catch per trap series and temperature of the original (upper) and filtered (lower) series. Values extending beyond the broken lines are significant at the 0.05 level.

TABLE 3. Parameter estimate and associated statistics for a transfer function model relating 1930–85 catch (kg) per trap with water temperature during year of capture. T is the t-ratio for the parameter, Q is the χ^2 statistic for autocorrelation on the residuals (through lag 24), and D is the Kolmogorov–Smirnov statistic for normality applied to the model residuals. Signs of the coefficients follow standard Box–Jenkins notation with use of the backshift operator. *Significant at $p < 0.05$; **nonsignificant at $p > 0.05$.

Parameter	Lag	Estimate	SE	T	Q	D
ω_0	0	0.861	0.260	3.32*	14.20*	0.094*
ω_1	1	1.067	0.259	4.11		

where all terms are defined as before. Comparison of the crosscorrelation of the original (unfiltered) series and that of the filtered series is particularly instructive (Fig. 4). The crosscorrelation of the original series is characterized by large positive correlations centered at a lag of 6 yr and no significant temperature effect at a lag of 0. In contrast, the crosscorrelation of the filtered series does indicate an immediate temperature effect in addition to a lesser effect with a delay of 6 yr. Temperature therefore appears to exert an immediate effect on catchability or availability and a delayed effect which may reflect an increase in survival during the early life history stages.

A transfer function model incorporating the temperature effects at lags of 0 and 6 yr was specified and the noise component was modelled based on the autocorrelation and partial autocorrelation functions of the residuals of the preliminary model. A first-order autoregressive term was used to model the noise component (Table 2). Incorporation of the temperature term resulted in a 27% reduction in residual variance relative to the univariate model. A comparison between the fitted transfer function model and the observed 1945–85 series is provided in Fig. 5. The predicted 1986 catch level was 8685 Mt; despite

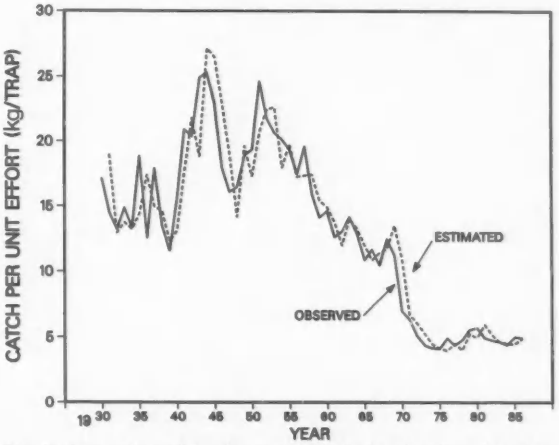

FIG. 7. Comparison of the observed (solid line) and estimated (broken line) catch per trap series for the period 1930–85. Observed and predicted catch per trap levels for 1986 are also provided.

the improvement in fit relative to the univariate model, the transfer function model provided a slightly less accurate forecast for 1986.

Catch-per-Unit-Effort (1930–86)

The catch (kilograms) per trap series was clearly nonstationary in level and first differences were taken. The autocorrelation and partial autocorrelation coefficients of the series indicated that the differenced series followed a random walk (i.e. differencing the log-transformed series resulted in a white noise process). The best naive prediction of CPUE is therefore simply the CPUE in the preceding year.

For development of a transfer function model, the temperature series (1930–85) was first modelled and used to filter both series. The temperature model was similar to the previous temperature models:

$$(1 - 0.473B - 0.269B^2)(\ln T_t - \mu) = a_t$$

where all terms are defined as before. The crosscorrelation between the filtered catch per trap and temperature series again indicated significant correlations at lags of 0 and 1 yr (Fig. 6). In contrast, the crosscorrelation coefficients for the original series indicated significant correlations only at high lags with no obvious biological interpretation. The immediate temperature effects for the filtered series (Fig. 6; Table 3) is consistent with previous results. The observed and fitted CPUE series are illustrated in Fig. 7. The predicted 1986 catch per trap of 4.87 kg compared favorably with the observed level of 4.94 kg.

Monthly Catch (1968–86)

A clearly defined seasonal pattern was observed in the monthly catch series (Fig. 8). Catches typically peaked during September–October. Interestingly, a minor peak often occurred in May. Reduced catchability during the June molting period may result in depressed catches relative to May levels.

The undamped sinusoidal pattern of the autocorrelation function for the monthly catch series indicated the need for seasonal differencing with a period of 12 mo. Further differencing and transformation was found to be unnecessary. Autocorrelation

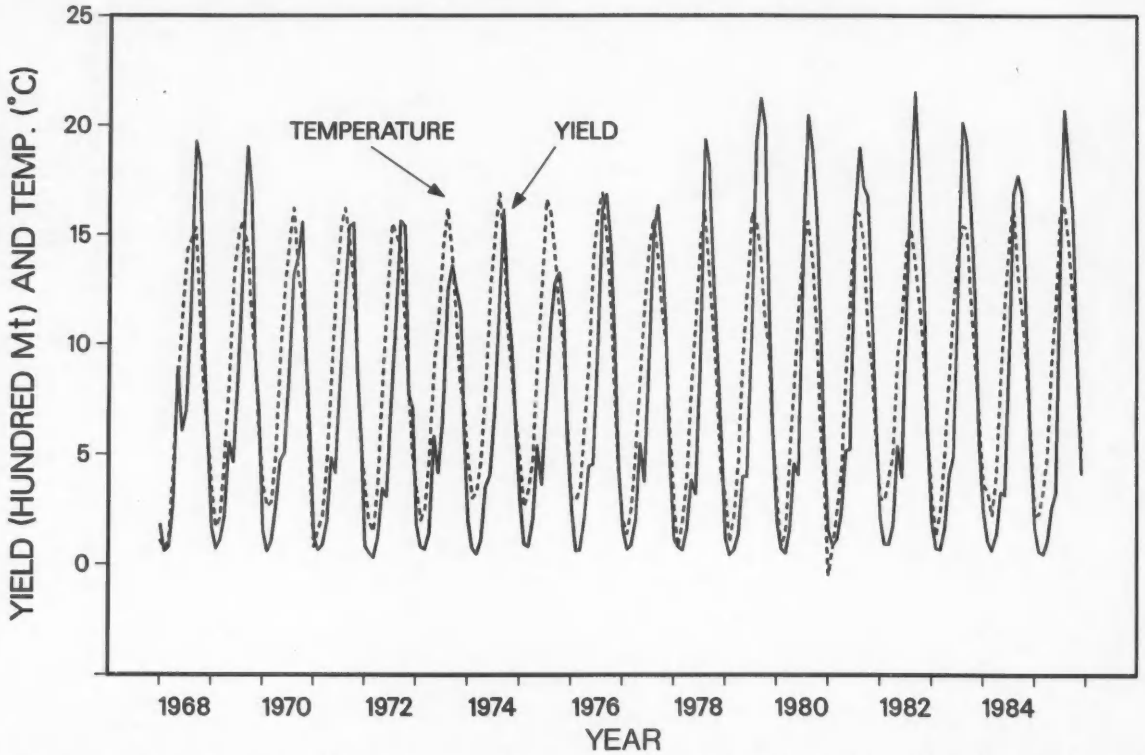

FIG. 8. Monthly Maine lobster yield (solid line) and mean monthly water temperature (broken line) at Boothbay Harbor, Maine, for the period 1968–85.

TABLE 4. Parameter estimates and associated statistics for a univariate autoregressive model for monthly lobster catches during 1968–85. T is the t-ratio for the parameter, Q is the χ^2 statistic for autocorrelation in the residuals (through lag 30), and D is the Kolmogorov–Smirnov statistic for normality applied to the model residuals. Signs of the coefficients follow standard Box–Jenkins notation with use of the backshift operator. *Significant at $p < 0.05$; **nonsignificant at $p > 0.05$.

Parameter	Lag	Estimate	SE	T	Q	D
ϕ_1	1	0.483	0.061	7.86*	41.75**	0.057**
Φ_1	12	−0.421	0.065	−6.53*		

and partial autocorrelation functions of the seasonally differenced series indicated an autoregressive model of the form

$$(1 - \phi B)(1 - \Phi B^{12})(1 - B^{12})(\ln C_t) = a_t.$$

Examination of the model residuals indicated no departure from the assumptions of independence (Table 4). Forecast values of the combined monthly catch for 1986 differed from the actual 1986 total catch by less than 3%.

Following seasonal differencing of the temperature series, the autoregressive model

$$(1 - 0.254)(1 + 0.598B^{12} + 0.314B^{24}) \times (1 - B^{12})(\ln T_t) = a_t$$

was obtained. The filtered monthly catch and temperature series were then crosscorrelated. Crosscorrelations between the original series were high, reflecting the periodicity of the undifferenced catch and temperature series (Fig. 9). A significant cross-correlation coefficient was obtained for the filtered series at a lag of 2 mo; however, further testing indicated that including a temperature term at this lag did not improve the model. Accordingly, development of a transfer function model for the monthly series was not pursued further. The lack of a significant temperature effect for the monthly catch series undoubtedly reflects the fact that both series are characterized by a strong seasonal cycle and there is little additional information in the temperature series relative to the catch series. This result does not imply that temperature has no effect on monthly lobster catch. Observed and fitted catch data for the period January 1968 to December 1985 based on the univariate model are illustrated in Fig. 10; observed and forecast values for January–December 1986 are depicted in Fig. 11.

Discussion

The principal objective of this study was to evaluate the potential role of temperature in influencing catch and CPUE levels in the Maine lobster fishery. Development of transfer function models provided useful insights into the relationship between water temperature and Maine lobster yield. In particular, the approach of prewhitening the catch and temperature series and then crosscorrelating the filtered series resulted in a clear definition of immediate temperature effects. This immediate effect was obscured in some of the relationships for the unfiltered series. The correlation between water temperature and annual yield or CPUE after filtering was considerably less than that for the original series. In part, this may reflect the fact

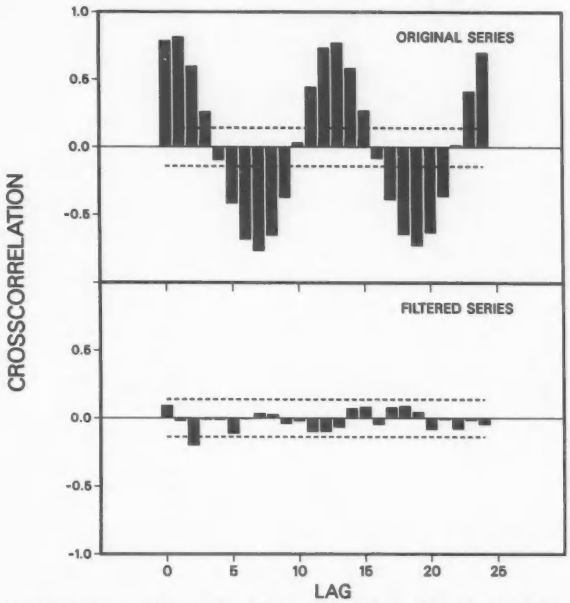

FIG. 9. Crosscorrelation between monthly lobster yield and temperature for the original (upper) and prewhitened (lower) series. Values extending beyond the broken lines are statistically significant at the 0.05 level.

that prewhitening is generally conservative (Box and Pierce 1970). This method does, however, greatly reduce the probability of obtaining spurious relationships, particularly between nonstationary series.

Previous studies have focused on delayed temperature effects on Maine lobster yield (e.g. Dow 1976, 1977, 1978); however, Flowers and Saila (1972) and Orach-Meza and Saila (1978) considered immediate and delayed temperature effects in their analysis of the Maine lobster fishery. The results of the present study using the annual catch during 1945–85 are generally consistent with those of Flowers and Saila (1972) and Orach-Meza and Saila (1978). Marchesseault (1977) demonstrated a temperature effect at a lag of zero for the Massachusetts lobster fishery but noted that delayed temperature effects were not well defined.

Maine lobster landings underwent a dramatic increase during 1942–45. The minimum legal size of lobster was raised in 1942 (Thomas 1973). This change should result in a short-term decrease in supply followed by a long-term gain (assuming constant effort). The effect of the Second World War on fishing patterns presumably also had an effect on yield. These impacts can be modelled using intervention analysis (Box and Tiao 1975). I developed preliminary intervention models for the Maine lobster fishery but these did not result in more accurate forecasts or help in interpreting the relationship between catch and water temperature. Accordingly, further development of intervention models was not pursued. It is clear, however, that the rapid change in catch during 1942–45 affects both development of time series models for the 1928–85 annual catch series and inferences about the relationship between temperature and yield for this period. The 1945–85 series, in contrast, is stationary and interpretation of the temperature–catch relationship is less problematical.

Mechanisms underlying proposed environmental effects on fish or invertebrate populations should be clearly identified. Direct temperature effects on lobster yield can be reasonably postulated. Temperature directly influences activity levels and hence catchability of the American lobster (McLeese and Wilder 1958; Paloheimo 1963). Short-term increases in yield therefore may accompany increased temperature levels. Further, increased annual probability of molting of sublegal-sized lobsters has been associated with increased water temperature (Ennis et al. 1982); short-term increases in yield can therefore occur as a result of increased recruitment into the harvestable size range. The effects of elevated activity and growth levels would occur at short lag times (primarily 0–1 yr) and would have a direct effect on catch (assuming relatively constant levels of effective effort).

A significant temperature effect at a lag of 6 yr was also observed for the 1945–85 series. Earlier investigations suggested the importance of previous temperature levels with lags centered at about 6 yr (Flowers and Saila 1972; Dow 1977, 1978; Orach-Meza and Saila 1978). It has been inferred that this represents a temperature-mediated effect on larval or early juvenile survival, since the expected time to reach legal size is approximately 5–7 yr. A mechanistic interpretation of the temperature–yield relationships at a lag of 6 yr implicitly requires catch to be a proxy for recruitment. Total mortality rates for lobsters in coastal Maine waters are indeed extremely high (Anthony 1980) and recruits dominate the Maine lobster catch (Thomas et al. 1983). However, interannaul variation in mortality rates complicates interpretation of catch as an index of recruitment. Despite this source of observational error, further consideration of possible mechanisms underlying a lagged temperature effect on recruitment is instructive. Scarratt (1964) and Caddy (1979) have suggested that the pelagic larval phase must be completed prior to winter when further molting will be prohibited. Larvae that do not reach the benthic stage prior to winter do not survive. Molting rates are highly temperature dependent (Templeman 1936); therefore, increased temperature may increase the probability of successful completion of the larval phase. It is also possible that the larvae are particularly vulnerable during the pelagic larval phase and that mortality is related to the time required to complete this phase.

Time series models that incorporate temperature effects for specified critical time periods (e.g. the first several months of life) could have higher resolution than those described here. Models of this type should be based on clearly stated a priori hypotheses, however, and not extensive data searches in which a large number of possible monthly combinations are considered. The possibility of obtaining spurious relationshps is high if the latter strategy is followed (Gulland 1965; Ricker 1975; Sissenwine 1984).

Flowers and Saila (1972) developed several predictive multiple linear regression equations for the Maine lobster fishery based on combined mean annual temperature lagged 6–8 yr and mean temperature for the months of January–March at Boothbay Harbor. Equations were also developed from Nova Scotia lobster landings using various lagged mean annual or monthly water temperatures measured at Lurcher Lightship. The previous year's landings were included in one of these equations, anticipating the use of autoregressive terms in yield prediction equations. Orach-Meza and Saila (1978) extended the multiple regression approach to a polynomial distributed lag model in which the effect of the independent variable was distributed over time, with some fraction apportioned to each lag period.

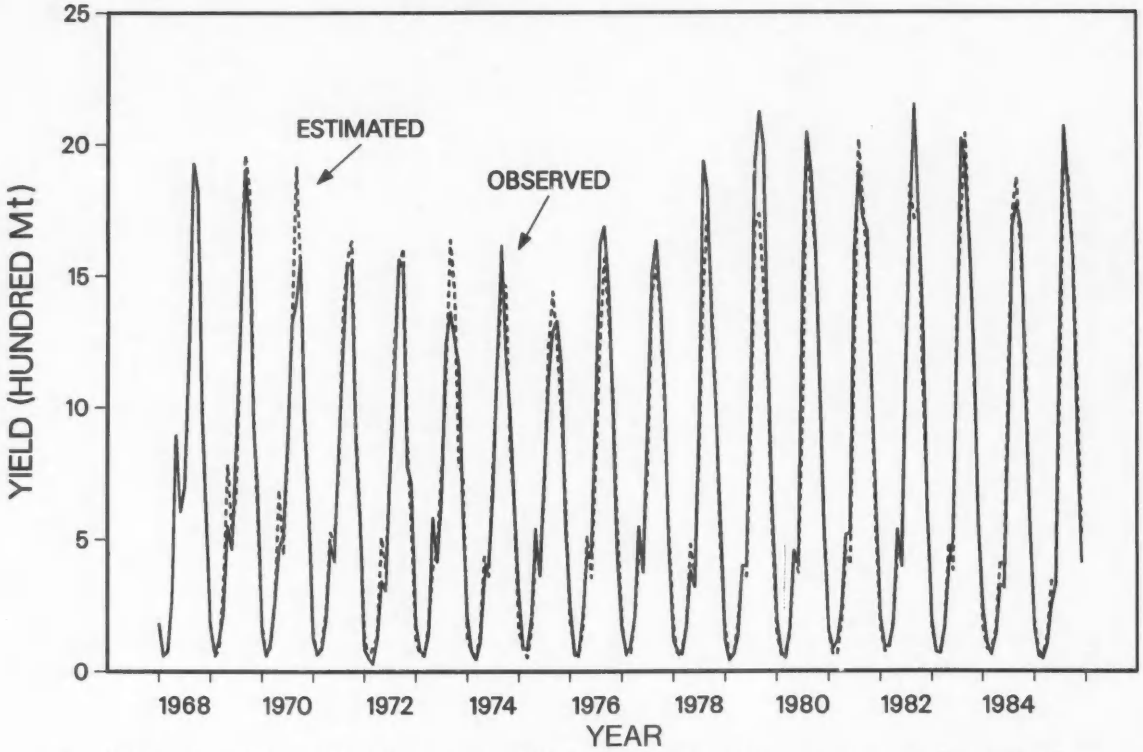

FIG. 10. Comparison of observed (solid line) and estimated (broken line) monthly lobster yield series for the period 1968–85.

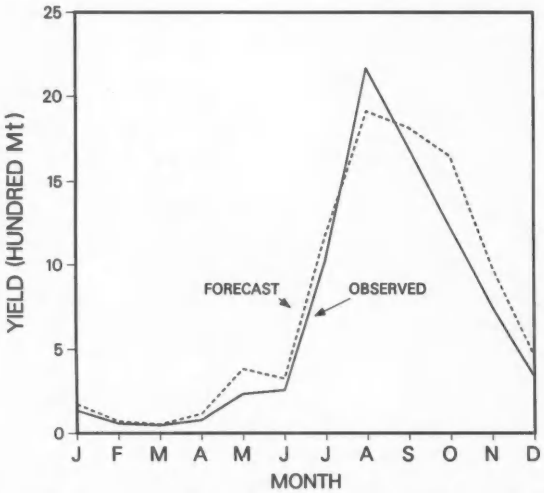

FIG. 11. Comparison of observed (solid line) and forecast (broken line) 1986 monthly lobster yield using the derived transfer function model.

In the present analysis, higher order transfer functions would be necessary to allow complex behavior of this type.

A secondary objective of this analysis was to evaluate the predictive capability of time series models for the Maine lobster fishery. Predicted 1986 catch and CPUE levels for the Maine lobster fishery differed by no more than 4% of the actual catch. Clearly, the relatively low interannual variability makes this fishery particularly suitable for developing reasonable forecasts. Inspection of the estimated and actual annual catch and CPUE series did indicate, however, that the models did not accurately predict rapid changes in landings. This result is not unexpected for autoregressive models; these changes were unrelated to changes in water temperature and could not be predicted based on the past history of the series.

Time series methods are a useful adjunct to standard fishery assessment methods (Saila et al. 1980; Mendelssohn 1981). The forecasting capability of autoregressive models compared with surplus production models has been considered in some detail (Stocker and Hilborn 1981; Roff 1983). It is clear, however, that these methods address quite different requirements. Surplus production models are used to model stock dynamics and to provide insight into the implications of alternative harvesting strategies. In contrast, time series methods do not explicitly model population processes and cannot provide information on optimal harvesting policies. Time series models can, however, provide valuable forecasts to guide short-term management decisions. Further, the Box–Jenkins modelling strategy provides a consistent and objective approach to developing empirical models and evaluating relationships among variables in complex systems.

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Effects of Liming on the Distribution of Cadmium in Water, Sediment, and Organisms in a Swedish Lake

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Andersson, P., and H. Borg. 1988. Effects of liming on the distribution of cadmium in water, sediment, and organisms in a Swedish lake. Can. J. Fish. Aquat. Sci. 45: 1154–1162.

We studied the cadmium concentrations in water, sediment, suspended particles, a free-swimming insect larva (*Chaoborus*), a sediment-bound insect larva (*Chironomus*), and liver of northern pike (*Esox lucius*) before and after liming operations in Lake Långsjön, Sweden. In accordance with the higher pH levels obtained in the lake water after the limings, cadmium concentration decreased in the water but increased in the sediment. Cadmium concentration in fish liver and *Chironomus* decreased after the limings whereas the concentration in *Chaoborus* larvae increased after the first liming. Possible mechanisms are discussed.

Nous avons déterminé les teneurs en cadmium de l'eau, des sédiments, des particules en suspension, d'une larve d'insecte libre (*Chaoborus*), d'une larve d'insecte limitée aux sédiments (*Chironomus*) et du foie du grand brochet (*Esox lucius*) avant et après le chaulage du lac Långsjön (Suède). Conformes aux pH élevés du milieu lacustre observés après le chaulage, les concentrations de cadmium ont diminué dans le milieu aqueux tandis qu'elles ont augmenté dans le sédiment. Les concentrations de cadmium dans le foie du grand brochet et chez *Chironomus* ont baissé après le chaulage tandis que la concentration dans les larves de *Chaoborus* a augmenté après le premier chaulage. Nous examinons des mécanismes possibles.

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During recent decades, a great number of lakes and waterways in Europe and North America have been acidified because of increasingly acidic precipitation (Environment '82 Committee 1982; Dickson 1983; Husar and Holloway 1983; Cowling 1983; Dillon 1983; Rehnberg and Wallin 1985). As a consequence of the acid-induced retention of metals in the water column and increased leaching from the watershed, increased concentrations of metals such as Al, Mn, Zn, Pb, and Cd have been observed in acidified waters (Dickson 1975; Henriksen and Wright 1978; Borg 1983, 1987a). The long-range atmospheric transport of heavy metals can also contribute to the observed increased levels of metals in lake water and sediments (Johansson 1980; Jeffries and Snyder 1981; Evans et al. 1983; Pacyna et al. 1984). A decreased pH generally results in a decreased sorption of metals to particles and a decreased complexation to organic substances. Relatively high proportions of dissolved species of Cd, Al, Pb, and Mn in acid lake waters have also been demonstrated (Borg and Andersson 1984; Borg 1987b). The changed metal speciation in acid waters probably influences the bioavailability and toxicity of the metals. An increased accumulation of Cd in mussels at decreased pH has been observed, especially in lakes low in humic substances (Campbell and Evans 1987), as well as a decreased toxicity to phytoplankton (Campbell and Stokes 1985).

As a countermeasure to the effect of acid precipitation, some 3000 of the 18 000 severely acidified lakes in Sweden have been treated with lime in recent years (Lessmark and Thörnelöf 1986). The aim of this study was to follow the effect of liming on the distribution of Cd in the water column, interstitial water, sediment, invertebrates, and fish. Parallel studies in the same area have been made concerning the effects of liming on concentrations of Hg in fish (Andersson and Kärrhage 1984) and on the aquatic community in general (Eriksson et al. 1982).

Description of Study Area and Limings

Lake Långsjön is situated downstream from Lake Trehörningen in the River Åvaån system approximately 25 km southeast of Stockholm (Fig. 1). The hydrological characteristics of the lakes are as follows:

	Lake Trehörningen	Lake Långsjön
Area (ha)	3.5	9.6
Max. depth (m)	4.4	8.0
Average depth (m)	2.4	4.0
Retention time (mo)	5	10

Lakes Långsjön and Trehörningen were limed on the ice and on land along the shore zone in March 1978. Altogether, 30 tonnes of lime was applied to the two lakes. During the reliming in October 1981 the lime accidentally became unevenly distributed. Consequently, the liming was repeated in late September 1982 when most of the dose was placed directly in the lakes.

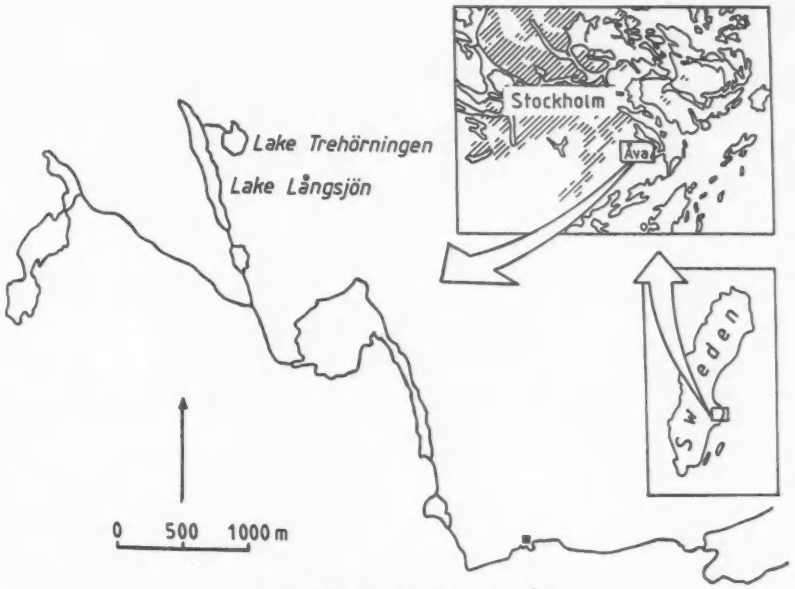

FIG. 1. Water system of the River Åvaån.

The total dose for the period 1981–82 was 56 tonnes. The lime used was $CaCO_3$ in the 0–0.2 mm fraction.

Methods

Measurements of pH, alkalinity, Ca concentration, sulphate concentration, and Cd concentration were made during the period autumn 1976 to autumn 1984. The pH of the sediment was measured at least once each year. The Cd concentration of the surface sediment was measured on five occasions during the period. The deposition of Cd to the sediment was studied annually from 1978. The concentration of Cd in fish liver was determined on northern pike (*Esox lucius*) caught in June 1977 and in late August during 1979–81, as well as during April–May 1985. Planktonic phantom midge larvae (*Chaoborus flavicans*) and sediment-bound chironomus larvae (*Chironomus anthracinus*) were sampled twice a year during November (week 45) and May (week 19) starting in 1977.

Water samples from the surface (0.5 m depth) and near-bottom (0.5 m above the bottom) at the greatest depth in the lake were taken for determinations of pH, alkalinity, Ca concentration, and sulphate concentration with a Ruttner sampler of Plexiglas and stored in polyethylene bottles. pH was determined in the laboratory immediately after arrival using a Radiometer refillable glass electrode. Alkalinity was determined by titration with hydrochloric acid to pH 5.4 with N_2 bubbling to eliminate produced CO_2, and Ca was determined by flame atomic absorption spectroscopy (AAS). Sulphate was determined by titration with barium chloride and Thorin indicator with photometric endpoint determination. The procedures followed Swedish standard methods (Svensk Standard, Series SS0281, Box 3295, 103 66 Stockholm, Sweden). Samples for determination of Cd in water were taken with the same sampler and stored in acid-washed polypropylene bottles (Nalgene). Prior to use, these bottles were soaked in 6 M HCl at 60°C for 3 d. They were kept totally immersed in the acid so that the outside was also cleaned. Then they were rinsed with Milli-Q water and filled with 0.05 M HNO_3, prepared from subboiling distilled ultrapure HNO_3, and Milli-Q water. The bottles were allowed to stand with the dilute acid until use, when they were rinsed several times with Milli-Q water. The bottles were kept in double plastic bags from the soaking in dilute acid until return to the laboratory, when the outer bag was discarded. All water samples for Cd determination were preserved by addition of ultrapure HNO_3 (2 $mL \cdot L^{-1}$) using Finnpipette with acid-washed polypropylene tips.

Cd was determined by graphite furnace AAS (Perkin Elmer 305B, HGA 74 with deuterium background correction). The graphite furnace was equipped with a fibre optics temperature control system (design LL Electronics, Bygdsiljum, Sweden) which allowed a more exact temperature control during ashing and atomisation and better precision in the Cd determinations. To further increase sensitivity and precision, the water samples were preconcentrated about 10 times in the sampling bottles by freeze-drying. Such a preconcentration was found to give accurate results with no problems with matrix interferences when $NH_4H_2PO_4$ was added as matrix modifier. Sampling, washing procedures, and determinations are further described in Borg et al. (1984).

Samples of sediment for determinations of pH, Cd concentration, and dissolved Cd in the porewater were taken in 1- and 2-cm layers, down to the 10-cm level, and placed in 500-mL polyethylene vessels using a Willner core-sampler of Plexiglas. Three to five cores were taken at the greatest depth in the lake. The pH was measured on gently homogenized subsamples of sediment within 24 h. The pH-KCl of the sediment was measured on 25-mL subsamples diluted with equal amounts of deionized water to which 5 mL of saturated KCl solution was added and then shaken for 2 h and sedimented for 24 h at 25°C. The Cd concentration of the sediment was determined by flame AAS following freeze-drying and digestion in 7 M HNO_3 in closed pyrex bottles in an autoclave (Swedish Standard

SS028150). Results on a dry weight basis were corrected for water content in the freeze-dried samples by drying an aliquot at 105°C for 24 h.

The interstitial water of the sediment was obtained by centrifuging (3500 rpm for 30 min) and vacuum filtration through a Nuclepore filter (0.4 μm) mounted in a polycarbonate filter holder. The filtrate was collected directly in polypropylene bottles. Preservation and determination of Cd was performed as for the lake water samples.

Sedimenting particles were collected in sedimentation traps with 20 polystyrene vessels (height 8 cm and bottom area 33.2 cm^2) mounted on a framework of PVC tubes placed 0.5 m above the bottom. The contents of the 20 vessels were freeze-dried and weighed individually whereas the Cd content was determined on a pooled sample from all sedimentation vessels. The determination of Cd was the same as for sediment.

On each occasion, one to eight juvenile pike with weights up to 100 g were caught, in connection with rotenone treatments in 1977, and on later occasions using electro-fishing units, fyke nets, and conventional nets. The fish were weighed and stored deep-frozen before their livers were removed. Cd concentrations in the individual livers were determined using the same graphite furnace AAS system as for the water samples, after freeze-drying and digestion with $HNO_3 + H_2O_2$ (Borg et al. 1981).

The chaoborids and the chironomids were collected using a dip net (mesh size 0.6 mm) drawn along the lake bottom at the deepest part of the lake, where it became filled with sediment which was removed by sieving (mesh size 0.6 mm). The sieving residues were kept in water-filled buckets before the larvae were transferred to Erlenmeyer flasks containing lake water. As the chironomid larvae frequently have large quantities of sediment and detritus in their guts, they were kept in flasks for 1–5 d at 5–8°C with weak aeration in order to empty their guts. The chaoborid and chironomid larvae were then blotted dry on filter paper and sealed in 2-mL quartz ampoules for determination of Cd by neutron activation analysis (Institute of Energy Technology, Norway). For each series, 10–15 individuals were preserved in ethanol for species determination.

The detection limit for Cd determinations in the preconcentrated water samples was around 0.005 $\mu g \cdot L^{-1}$. The blank values in the polypropylene bottles were always below the detection limit. The precision of the measurements at the levels of Cd in the actual water samples, generally more than 10 times the detection limit, was 2–7%. The accuracy of the Cd determinations in the fish livers was checked by analysis of NBS standard reference material 1577 (bovine liver) and by parallel analysis by neutron activation. The results were within the certified range (mean values differed 6.9% from NBS certified mean) and the precision for replicate digests was 7% (Borg et al. 1981).

Statistical significance of observed differences during the study period was evaluated using *t*-tests.

Results

pH, Alkalinity, and Ca and Sulphate Concentrations in the Water

The third liming in Lake Långsjön (Fig. 2) gave a slightly higher pH value and substantially higher alkalinity and Ca values than the two earlier limings. The pH value varied more in the surface water than in the bottom water depending on the temporary influx of acid water from the surroundings. Alkalinity and Ca concentrations were higher in the bottom water than in the surface water after the second and third limings.

The highest sulphate concentrations were measured in the years subsequent to the extremely dry years of 1976 and 1983, i.e. 1977 and 1984. The sulphate concentration in Lake Långsjön varied little during the study period, but showed some seasonal variation, with the highest values being recorded during the late winter to early spring period. The limings do not appear to have influenced the sulphur metabolism in the lake in any substantial way.

pH and pH–KCl in the Sediment

The pH in the surface sediment (0–2 cm) showed minor variations after the first liming and increased markedly after the third liming ($p < 0.05$) (Fig. 3). At deeper sediment layers the pH level generally was about 0.5 unit higher than at the surface and was less influenced by the lime treatments. The pH–KCl was about 1 unit lower than the pH and increased markedly only after the third liming ($p < 0.005$) when it reached higher levels in the surface sediment than at greater depths (Fig. 3).

Cd Concentrations in the Water and in the Porewater of the Sediment

Before the first liming, Cd concentrations were 0.15–0.35 $\mu g \cdot L^{-1}$ in the surface water and 0.10–0.15 $\mu g \cdot L^{-1}$ in the bottom water (Fig. 4). After the first liming the corresponding concentrations were 0.05–0.25 and 0.02–0.20 $\mu g \cdot L^{-1}$, respectively. Apart from a high value recorded in the autumn of 1978, decreasing concentrations were generally found during the first 2 yr after the liming in 1978 ($p < 0.005$). However, in the third year, high concentrations were again measured, particularly in the bottom water. After the second and third limings, lower concentrations in both the surface and the bottom were noted ($p < 0.005$), the lowest levels being 0.01 $\mu g \cdot L^{-1}$.

As also found for pH, alkalinity, and Ca, the variations in Cd concentrations were larger in the surface water than in the bottom water. The highest concentrations in the surface water were found in connection with the circulation periods in spring and autumn.

The concentration of dissolved (filterable) Cd in the porewater was markedly higher in the uppermost layer of sediment (0–1 cm) during the year after the first liming than previously (Fig. 5). On that occasion the concentration was as high or higher than the total concentration in the bottom water and considerably higher than in the porewater below (1–2 cm).

Cd Concentrations in the Surface Sediment and Sedimenting Particles

The Cd concentration in the surface sediment increased from slightly below 2 $mg \cdot kg^{-1}$ dw just before the first liming to almost 3 $mg \cdot kg^{-1}$ dw in the following year (Fig. 6a). Subsequently, the concentration decreased continuously until the second liming, 2 yr after which it was again at the 2 $mg \cdot kg^{-1}$ dw level. In the year after the first liming, the concentration of Cd in sedimenting particles collected in sediment traps was lower than in the surface sediment but thereafter was of the same magnitude as in the sediment (Fig. 6b). After the more intensive liming in 1981–82 the concentration of Cd in the particulate material was considerably higher (3.5–4.5 $mg \cdot kg^{-1}$ dw) than earlier and higher than in the surface sediment.

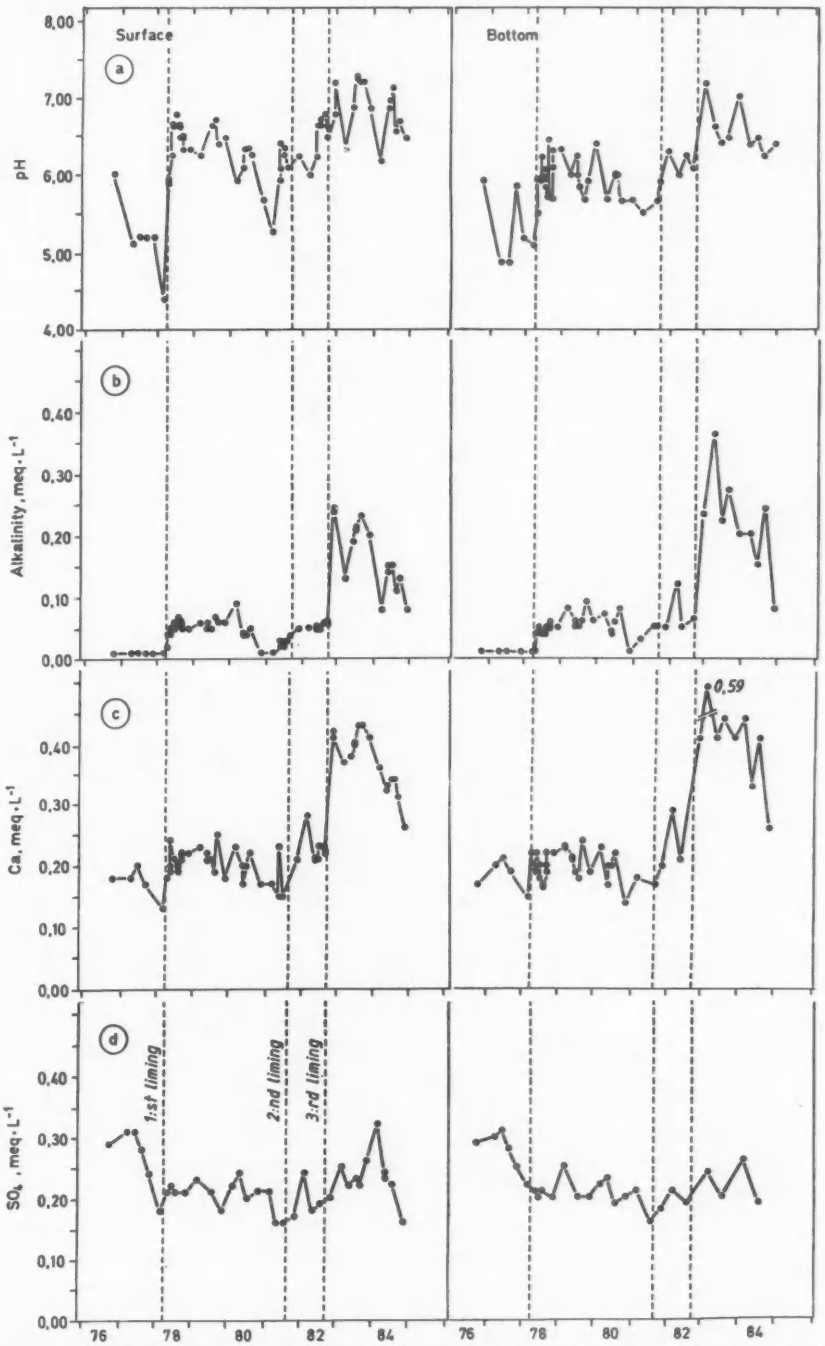

FIG. 2. pH, alkalinity, and Ca and sulphate concentrations in the surface water and bottom water of Lake Långsjön during 1976–84.

The sedimentation rate of Cd largely followed the same pattern as the concentration in sedimenting material. The largest deposition of Cd to the sediment (6 $\mu g \cdot m^{-2} \cdot wk^{-1}$) was obtained during 1983 following the third liming when the pH was around 7 and the alkalinity about 0.20 $meq \cdot L^{-1}$. During this period the lowest total concentration of Cd (0.01 $\mu g \cdot L^{-1}$) was measured in the surface and bottom water.

Cadmium Concentrations in the Biota

Mean concentrations of Cd in juvenile pike were 0.82

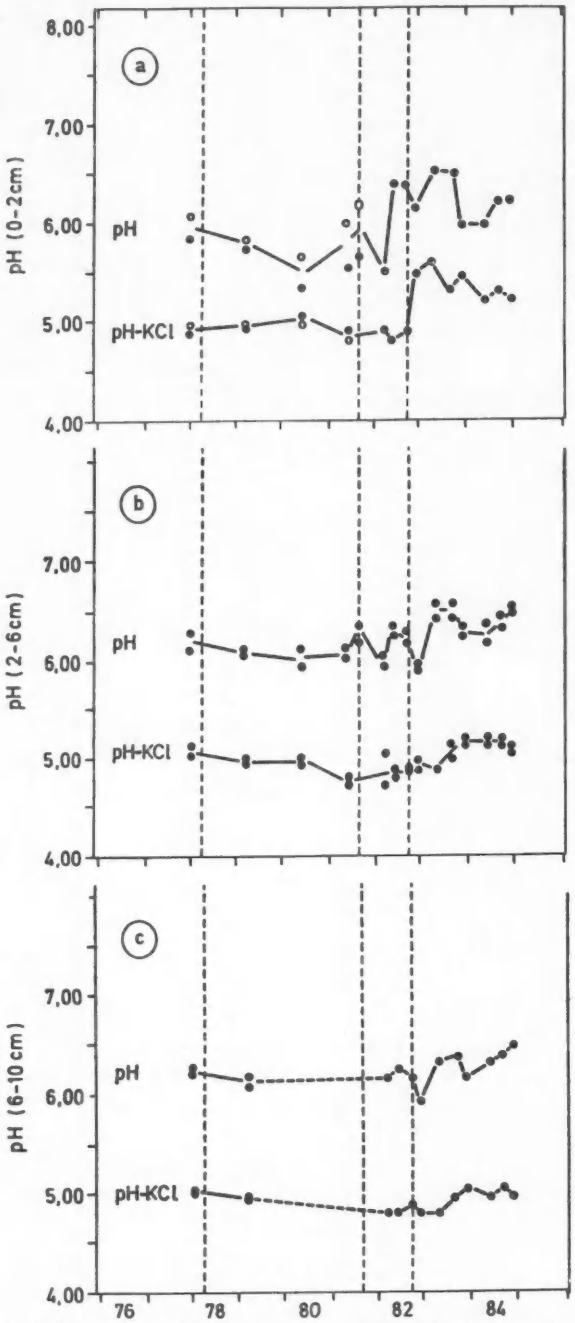

FIG. 3. pH and pH–KCl in the sediment at the deepest place in Lake Långsjön at levels of (a) 0–1 (●), 1–2 (○), 0–2 (⊙), (b) 2–6, and (c) 6–10 cm during 1977–84.

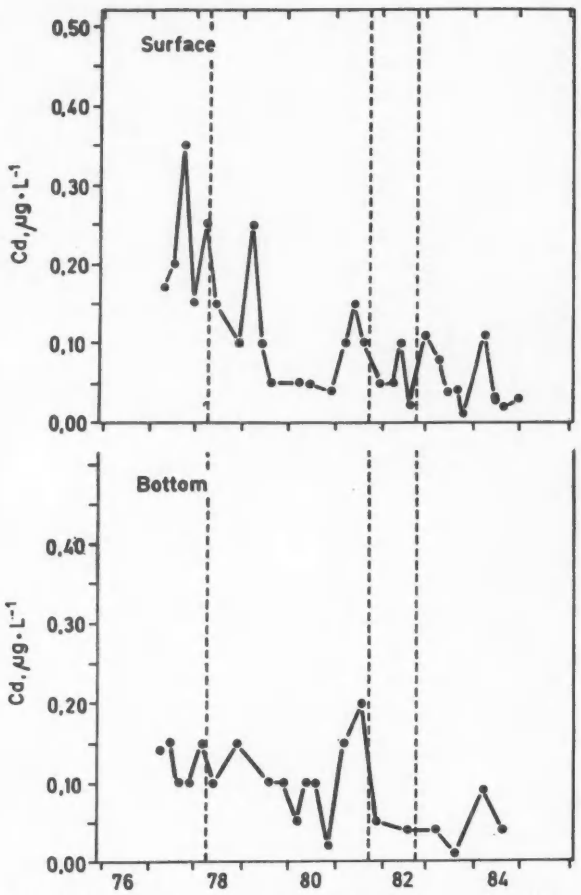

FIG. 4. Cd concentration in the surface water and bottom water in Lake Långsjön during 1977–84.

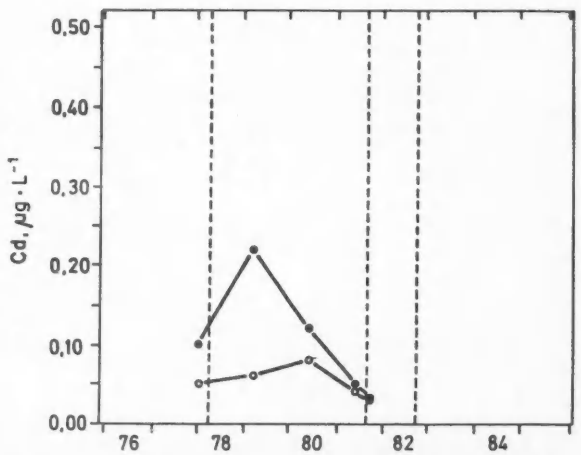

FIG. 5. Concentration of dissolved (0.4 µm) Cd in porewater at 0–1 (●) and 1–2 cm (○) of the sediment in Lake Långsjön during 1978–81.

mg·kg^{-1} ww before the limings and 0.3–0.5 mg·kg^{-1} ww after the first liming. Two years after the third liming the Cd concentration still was only half as high as before the first liming. The decrease from 1977 to 1980 was significant ($p < 0.01$) as was that to 1985 ($p < 0.05$). In accordance with the reacidifi-

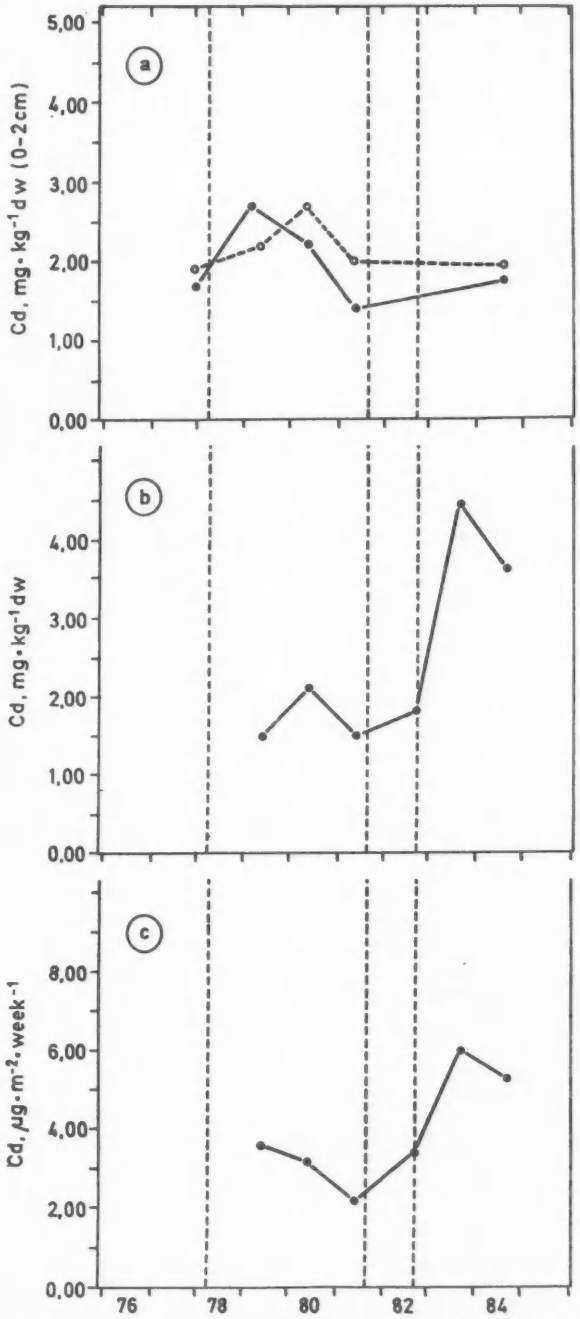

FIG. 6. Cd concentration in the surface sediment at (a) 0–1 (●) and 1–2 cm (○) and (b) sedimenting material and (c) Cd deposition in Lake Långsjön during 1978–84.

cation after the first liming, the Cd level had again increased somewhat in 1981 and was no longer significantly lower than in 1977 ($p < 0.25$). The difference between pike caught before liming and all pike caught after liming was, however, significant ($p < 0.05$) (Fig. 7).

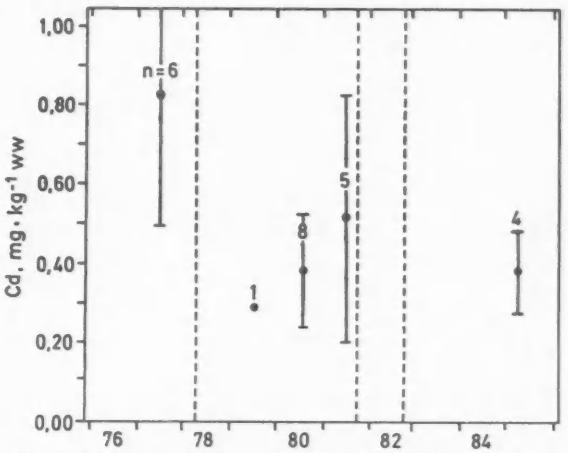

FIG. 7. Cd concentration (mean ± standard deviation) in liver of pikes in the size range 0–100 g in Lake Långsjön during 1977–85.

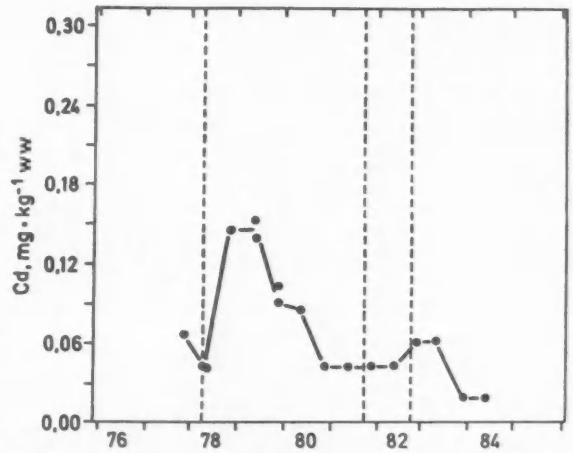

FIG. 8. Cd concentration in *Chaoborus flavicans* larvae from Lake Långsjön during 1977–84.

After the first liming, the Cd concentration in *Chaoborus* larvae increased from 0.06 to 0.15 mg·kg^{-1}(Fig. 8). It was not until 2 yr later that the concentration had fallen back to the same level as before the liming. After the two latest limings there was only a slight increase in the concentration for about 6 mo, after which the levels were the lowest found during the entire period of the investigation. The concentration of Cd in sediment-bound *Chironomus* larvae showed a general decrease throughout the study period (Fig. 9). The three replicate samples in 1977 represent different size classes of *Chironomus* (10, 13, and 18 mg as mean individual wet weights), with the highest Cd concentration in the largest individuals. To keep the size influence on Cd to a minimum in the following sampling occasions, the total weight range was kept within 10–16 (12.1 ± 2.7) mg with one exception (18 mg) in autumn 1983 (Fig. 9).

Discussion

The Cd concentrations measured in the water of Lake Långsjön were frequently higher and periodically considerably higher

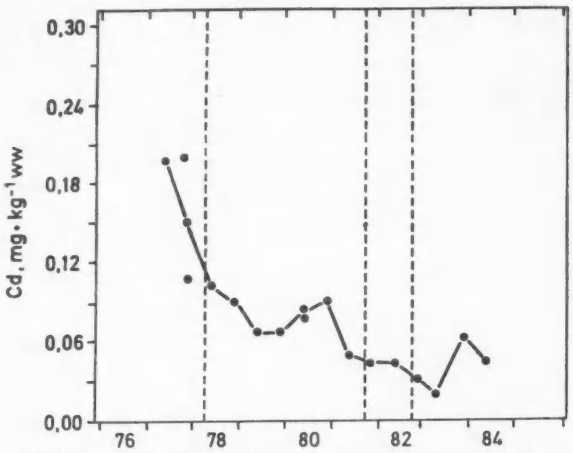

FIG. 9. Cd concentration in *Chironomus anthracinus* larvae from Lake Långsjön during 1977–84.

than those measured in other Swedish forest lakes (Borg 1984, 1987a). This also applies to other lakes in the Åva area and is probably associated with their proximity to Stockholm. In addition, the area is dominated by bedrock with thin layers of soil with poor buffering capacity which could favour the transport of Cd deposited in the catchment area to the lakes.

The reduction of Cd in the livers of the juvenile pikes (Fig. 7) after the first liming was expected because the concentration in the water, and therefore exposure of the fish, decreased. A higher pH level usually means both a lower total concentration of Cd and a decreased proportion of dissolved Cd in the water (Dickson 1979, 1980; Borg 1983; Borg and Andersson 1984), a condition that would probably cause a decreased bioavailability of Cd. The increased water hardness after the limings might also contribute to a decreased bioaccumulation of Cd (Campbell and Evans 1987). However, other factors such as increased growth rate after the liming caused by a decreased acid stress might also be of importance for the accumulation pattern of Cd in fish liver (Bendell-Young et al. 1986).

After the limings, there was a higher sedimentation rate of Cd to the bottom than previously. The deposition rate measured in the sedimentation traps ranged from 2 to 6 $\mu g \cdot m^{-2} \cdot wk^{-1}$. Those figures are in good agreement with other reported accumulation rates for Cd in lake sediments. Rehnberg (1986) measured an anthropogenic accumulation rate of 2.9–5.8 $\mu g \cdot m^{-2} \cdot wk^{-1}$ in varved lake sediments in northern Sweden. Johnson et al. (1986) reported 1.9 $\mu g \cdot m^{-2} \cdot wk^{-1}$ as background and 3.9 as surface sediment mean loading rate for lakes in southern Ontario. The total amount of Cd to which *Chaoborus* was exposed might have increased directly after the addition of lime. However, Cd concentrations in *Chaoborus* reached the highest levels after the first and weakest lime treatment. Consequently, it was probably not the increased total amount of Cd which was the decisive factor for the concentration level in *Chaoborus*. The increased accumulation might instead be a result of increased mobility of Cd at the sediment surface. The supply of calcium ions to the bottom probably caused a release of former bound cadmium and hydrogen ions by ion exchange. The first lime dose, which was relatively low, was probably not enough to neutralize the released hydrogen ions in the near-bottom water and the porewater, a condition that favoured an increased mobility of Cd. The highest concentration of dissolved Cd in the porewater from the level 0–1 cm was measured at the same time as the highest level in *Chaoborus* was recorded. The lime dose at the second and third liming was considerably stronger than the first, so there was probably a sufficient amount of base present to cope with the released hydrogen ions. Only after the third liming could we register a significant ($p < 0.005$) increase in pH–KCl simultaneously with the increase in pH–"water." The total amount of hydrogen ions then had obviously decreased and there was only a minor increase in the Cd concentration in *Chaoborus*.

The Cd concentration in sediment-bound burrowing *Chironomus* larvae is probably influenced mostly by conditions in deeper layers of sediment in contrast with the Cd concentration in *Chaoborus* which is influenced by conditions in the bottom water and surface sediment. It is more difficult to explain concentration variations in *Chironomus* than *Chaoborus* because it is not fully clear where in the sediment *Chironomus* lives. The small changes in pH and pH–KCl at the 2–6 cm and 6–10 cm levels after the first liming give no indication that the Cd availability to *Chironomus* had varied.

The situation in the sediment is certainly more complex than can be explained with the variables used here. For example, the redox potential of the sediment can influence Cd mobility. The addition of lime might cause an increased degradation of organic matter and more reduced conditions, which would make Cd less soluble and less potentially available. Cd has been shown experimentally to be more soluble under acid oxidizing conditions than under nonacid reducing conditions. Even plant uptake of Cd was greatest at low pH (5.0) and in oxidized sediment (Patrick et al. 1977). An increased degradation in the sediment might also cause an increased oxidation of S compounds into sulphate, which would favour a pH decrease. The minor variation in sulphate levels recorded especially in the bottom water does not suggest, however, that these processes would be of importance (Fig. 2). The measured pH changes in the sediment after the limings was only small with the exception of the third liming. However, it is probable that the sediment samples were partly oxidized during sampling and sample handling, so the real pH might have been higher. The content of dissolved Cd in the porewater is of course also influenced by sample handling procedures, although it is difficult to be sure in what way. The pH decrease following oxidation would favour dissolved Cd species, but on the other hand, it also favours the formation of ferric and manganic oxides with which Cd and other metals might be coprecipitated (Patrick et al. 1977).

It is known that metals are released from soil during acidification (Tyler 1978, 1981). The release of metals from the sediments in acidified lakes is not as well known. In an acidic lake, lower concentrations of easily mobile metals such as Zn and Cd are usually seen in the recent sediment layers which have been formed since the lake became acid than in the older and deeper sediment layers (Johansson 1980; Salomons and Förstner 1984). The reason for these lower concentrations in the uppermost centimetres of the sediment is probably associated with an acid-induced increased retention of metals in dissolved form in the water column, rather than release of metals from the sediment surface. For some metals such as Zn and Cd, a net transport of metals downwards in the sediment might also be important (Carignan and Tessier 1985). However, a rapid release of metals from the sediment is obtained when a rapid pH reduction occurs; this has been demonstrated experimentally both in lakes (Schindler et al. 1980) and in the laboratory (Andersson et al. 1984).

Release of metals from sediment after liming has been observed in connection with limings in Sweden other than the one in Lake Långsjön. In Lake N. Särnamannasjön on the Fulufjället mountain area, for example, Cd content in the lake's bottom water increased 10-fold when the Cd in the surface sediment was released (Swedish Board of Fisheries and Swedish Environmental Protection Board 1981). It is probable, as in Lake Långsjön, that calcium ion exchange with hydrogen and cadmium ions occurred. With regard to the relatively low pH reduction in the sediment of Lake Långsjön, it is probable that direct ion exchange between Ca and Cd was of greatest importance. This is also supported by laboratory experiments made with sediment from Lake Trehörningen and Lake Långsjön, which demonstrated a release of Cd after small additions of Ca and negligible changes in pH (Andersson et al. 1984).

From our results it appears that there is a risk of Cd being released from the sediment following small additions of lime. Possible biological effects will probably be limited, however, to the organisms at the interface between the water and the sediment. If, on the other hand, the water in the lake is allowed to become more acid than the sediment, which occurs when the liming effect decreases and the lake again becomes acidified, there would be a risk that easily mobile and formerly sedimented Cd will enter the water column. This is exemplified by the increase in the concentration of Cd in the bottom water and the decrease in the concentration in the sediment in Lake Långsjön prior to the liming in 1981 (Fig. 4, 6). Other examples also demonstrate that a limed lake that has again become acidified has considerably higher Cd concentrations in the water than other lakes with the same pH level (Dickson 1982).

However, the problem with release of metals from sediment is limited to the near-bottom water and consequently a "local" problem as long as the pH level in the lake is kept high enough by repeated addition of lime, i.e. so high that the metals are precipitated back to the sediment. However, it cannot be excluded that even more pelagic organisms also receive increased Cd loads through dispersal via the food chain. There is thus reason to be observant of both long-term and short-term effects of Cd in acidified and limed lakes. When liming lakes it is clear that metal deposits in the sediments will increase successively as long as the supply from the catchment area and from precipitation continues unchanged or increases as a result of soil acidification. This implies that a limed lake will become a leaking source of metal when the lime effect decreases and the acidity again increases. Consequently, if a liming program has been started, it must be continued until the input of acidifying substances has been sufficiently reduced.

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Effect of Experimentally Increased Intraspecific Competition on Sedentary Adult Brown Trout (*Salmo trutta*) Movement and Stream Habitat Choice

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Heggenes, J. 1988. Effect of experimentally increased intraspecific competiton on sedentary adult brown trout (*Salmo trutta*) movement and stream habitat choice. Can. J. Fish. Aquat. Sci. 45: 1163–1172.

Movements and habitat choice of 19 brown trout (*Salmo trutta*) resident in a stream was monitored during one season, while in the next season the population was increased by introducing 130 wild brown trout captured further downstream. No differences in the movement patterns of resident trout at the two population densities, or of residents compared with nonresidents, were found. Median movement distance was 0 m. Sixty-six percent of the movements were less than ±50 m, 11% were between 50 and 100 m, and 23% were greater than ±100 m. Introduced nonresident trout occupied habitats different from those occupied by residents. Nonresidents were more often observed in shallower areas with less cover and finer substrate, i.e. less preferred habitats. It is hypothesized that this was caused by dominance of resident trout due to their larger size and prior residence. Both resident and introduced trout exhibited preferences for deep stream areas, e.g. water >50 cm deep, mean water velocities in the range 10–70 cm/s, coarse substrate >128 mm, and a >50% overhead cover compared with available habitat. At both low and high population densities, the trout inhabiting pools were significantly larger than trout inhabiting other habitat types. No homing tendency was observed for the displaced trout.

Les déplacements et le choix de l'habitat de 19 truites brunes (*Salmo trutta*) résidentes d'un cours d'eau ont été surveillés pendant une saison, tandis qu'au cours de la saison suivante la population était accrue par introduction de 130 truites brunes sauvages capturées plus en aval. Aucune différence n'a été relevée au niveau des profils de déplacement des truites résidentes pour les deux densités de population, ou entre les truites résidentes et les non résidentes. La distance médiane de déplacement était égale à 0 m. Soixante-six pour cent des déplacements couvraient une distance inférieure à ±50 m, 11 % une distance comprise entre 50 et 100 m, tandis que 23 % couvraient une distance supérieure à ±100 m. Les truites non résidentes introduites occupaient des habitats différents de ceux des truites résidentes. Les non-résidentes fréquentaient davantage des zones moins profondes comportant moins de couverture et un substrat plus fin, c'est-à-dire des habitats moins recherchés. On a émis l'hypothèse selon laquelle ce phénomène était causé par la dominance de truites résidentes à cause de leur grande taille et de leur résidence antérieure. Les truites résidentes et les truites introduites ont marqué des préférences pour des zones profondes, par exemple une profondeur de plus de 50 cm, une vitesse moyenne de l'eau de 10 à 70 cm/s, un substrat grossier de granulométrie supérieure à 128 mm et une couverture supérieure à 50 % par rapport à l'habitat disponible. Pour une densité de population faible et élevée, la truite fréquentant des étangs avait une taille significativement plus grande que celle qui fréquentait d'autres types d'habitats. Les truites déplacées ne présentaient aucune tendance à retourner dans leurs eaux d'origine.

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Brown trout (*Salmo trutta*) life history in streams can be divided into discrete stages with respect to movements and migrations: (1) a migration of spawning trout, (2) movement of juvenile trout from hatching to nursery areas, (3) movement of young trout from nursery to areas of adult growth, and (4) movements by adults (except spawning migrations) (Solomon and Templeton 1976; Huet and Timmermans 1979). The degree to which this movement or migration pattern is displayed will depend on the habitat available.

Some studies have indicated movement by adult brown trout to be limited, i.e. from some hundred metres to a few kilometres, except in the spawning season (Shuck 1945; Stefanich 1952), but there is a lack of knowledge as to what extent local movements of a few metres occur within streams. It has been suggested that territorial behavior and hierarchy affect the degree of movement (Gerking 1959). Density, i.e. increased intraspecific competition and movement, may be positively related, possibly above some threshold level as suggested by Jenkins (1969). Territorial behavior and hierarchy may also affect habitat choice. The social structure of brown trout in a pool has been described as a cost-minimizing, size-dependent, linear dominance hierarchy of individuals having overlapping home ranges (Bachman 1984). Fish size and prior residence have been suggested to be the dominant factors determining hierarchy structure for brown trout (Jenkins 1969; Bachman 1984). The mean size of the restricted home ranges may be presumed to vary with size of fish and habitat availability, and it may also be affected by introduction of nonresident trout.

The main purpose of this study was to investigate (1) the local movements of wild brown trout in a stream, (2) whether movements of the trout were influenced by an introduction of nonresidents and if nonresidents exhibited a different movement pattern, (3) whether habitat choice changed with an introduction of nonresident trout, and (4) what habitat preferences the

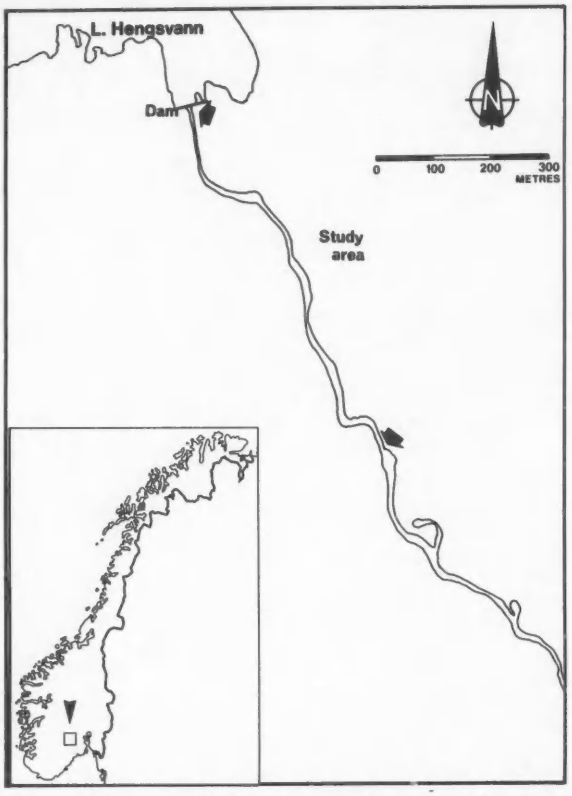

FIG. 1. Study area on the River Hengselva, downstream from Lake Hengsvann. Arrows indicate the extremities of the investigated stream reach. Inset shows location of the study stream in Norway.

brown trout had compared with total available habitat.

Since a homing tendency after spawning migrations and other displacements has been noted for brown trout in streams (Shuck 1945; Allen 1951; Stuart 1957; Solomon and Templeton 1976), a secondary objective of the study was to see if brown trout artificially displaced upstream from one stream section to another showed any homing behavior.

Study Area

A sedentary population of fish with well-defined distribution limits was necessary to minimize the possibility of fish movement beyond the limits of the sampling area. As well, the area had to be undisturbed by anglers to nullify any bias due to fish being removed from the stream.

A section of the River Hengselva, 10 km west of Kongsberg, southeast Norway, was chosen for the study (Fig. 1). Hengselva is a softwater stream rising in the reservoir Lake Hengsvann and flowing south through pine forests for 5 km to its confluence with the River Storelva. It varies in width from approximately 7 m downstream of Lake Hengsvann to approximately 12 m at its mouth. The average gradient is about 35 m/km. Riparian vegetation of pine forest along the stream is sparse. Brown trout is the only species in the stream. The population is sparse, but naturally reproducing.

A 780-m-long section of the stream immediately downstream of the reservoir outlet was investigated. At the reservoir outlet, a dam which was impassable for upstream migrants closed the upper end of the stream. Streamflow varied relatively little because of the reservoir. The study area ended downstream in a large pool with a shallow and wide outlet. Thus, no physical barrier prevented the trout from moving downstream, but the pool and outlet represented a pronounced habitat shift. The study area had an average gradient of 3.4 m/100 m and consisted of three distinct habitat zones (Table 1). The lower section was characterized by a few large pools and short riffles, while the middle section had a high gradient (7:100), high water velocities, and bottom substrate of boulders or bedrock interrupted by two pools. The upper section consisted of long riffles with many smaller pools and more variable, although coarse, substrate. The deepest pool in the study area was 1.2 m during normal water levels. The entire stream area could readily be sampled with an electrical shocker. There was virtually no angling in the area because of controlled access, and fishing mortality could be discounted.

Methods

The study area was divided into 26 segments, each 30 m long and numbered from 0 to 25, with segment 0 being furthest downstream. Within each segment, transects at 3-m intervals were marked in the field and numbered 0 through 9 consecutively from downstream to upstream. The marked 3-m intervals were also used as reference transects for habitat description. Brown trout were captured with a 1600-V AC electric stream shocker. Each captured fish was marked individually with a color marking code using a Pan Jet inoculator and Alcian blue (Hart and Pitcher 1969; Pitcher and Kennedy 1977), its position noted at the point where the captured fish was first observed (Hearn and Kynard 1986), its total length measured, and the fish released at the same position as where it was captured. To minimize any "fright bias" (Bovee and Cochnauer 1977), a specific survey procedure of "point" electrofishing was adopted. The anode was placed sequentially at maximum five equidistant points across the stream at each transect, rather than pushing it forward through the water between the transects. In this way, only the transects, which also were the reference points for habitat description, were systematically electrofished. Positions of observed fish were referred to station number, transect, and point across the transect, i.e. five observation "cells" were possible in each transect.

During 1985–86 the brown trout population in the stream was sampled at approximately 2-wk intervals from May to November. In July 1986 the population was increased by introducing 130 nonresident wild brown trout to the study area. They were captured at two different sites further downstream. Sixty-five of the introduced fish were captured at a 100-m-long site situated 1100 m downstream of the study area, while 65 more were captured in a 20-m-long and 3- to 8-m-wide pool located 3300 m downstream. The captured nonresident trout were held in a net for 48 h before they were marked individually and dispersed evenly along the entire study area. Two weeks elapsed after release before the next sampling occurred.

The introduced trout had a mean length of 17.0 cm (SE ± 0.23, $n = 130$) in the range 12.1–27.5 cm, while the residents' mean length was 24.7 (SE ± 0.23, $n = 19$) in the range 11.2–43.3 cm.

After July 1986 the sites from which introduced fish were captured were also checked for homing of individuals. The actual sites were repeatedly electrofished until no more fish

TABLE 1. Habitat characteristics of the study area and the three subsections.

Site	Length (m)	Mean width (m)	Mean depth (cm) (range)	Mean surface velocity (cm/s) (range)	Mean water velocity (cm/s) (range)	Mean cover (range)[a]	Dominant substrate (mm) (range)[b]
Entire study area	780	6.9	22.8 (0–120)	27.2 (0–225)	23.3 (0–240)	1.6 (0–9)	430 (2.1–bdr)
Lower section	190	8.7	21.3 (0–105)	24.6 (0–158)	19.9 (0–170)	1.3 (0–9)	330 (org–bdr)
Middle section	210	5.3	19.3 (0–86)	40.2 (0–225)	38.6 (0–240)	2.4 (0–9)	sm bdr (8.1–bdr)
Upper section	380	6.9	25.3 (0–120)	23.3 (0–178)	18.8 (0–165)	1.3 (0–9)	400 (0.1–bdr)

[a]Visually classified from 0 (no cover) to 9 (full cover).
[b]bdr = bedrock, org = organic fine materials, sm bdr = smoth bedrock.

TABLE 2. Stream substrate classification system, modified Wentworth scale (modified after Bain et al. 1985).

Substrate type	Size class (mm)	Code
Organic fine materials		1
Organic rough materials		2
Clay, silt	0.004–0.06	3
Sand	0.07–2	4
Fine gravel	2.1–8	5
Gravel	8.1–16	6
Pebble	16.1–64	7
Small cobble	64.1–128	8
Cobble	128.1–256	9
Boulder	256.1–512	10
Large boulders	>512.1	11
Smooth bedrock		12
Rough bedrock		13

were captured. In addition, a 200-m reach downstream and upstream from the sites was also sampled. Movements in this study were calculated as distance moved from last site of observation.

For quantifying total habitat available in the study area and describing each cell across the transects, habitat variables were measured at points every 60 cm across each transect. A lead-core rope was divided into 60-cm sections painted alternately black and white and stretched across the stream perpendicular to the current at each transect. In all, 2938 point measurements were made along 260 consecutive transects. At each point the following measurements were made: (1) mean water column velocity measured at 0.6 depth, (2) surface water velocity measured 1 cm below the surface, (3) depth, (4) width, (5) substrate, and (6) cover. Depths were measured to the nearest centimetre with a measuring rod. Water velocities were measured with a Schiltknecht Micro Mini Water type 642 w-m/l flowmeter with an 8-mm propeller. The dominant substrate fraction under each 60-cm rope segment was visually classified according to a modified Wenthworth scale (Table 2) (modified after Bain et al. 1985). A mean (index of coarseness) and standard deviation (index of heterogeneity) for each transect could be computed from the recorded code values (Bain et al. 1985). Due to this coding procedure, both substrate parameters were made continuous and could easily be compared with the associated habitat parameters of depth and velocity. Cover was visually classified on a scale from 0 (no cover) to 9 (full cover) and computed in a similar way to substrate. The definition of cover was (a) any submerged structure (except substrate) beneath which fish could be hidden from a view overhead, (b) undercut banks or overhanging vegetation <50 cm above water surface, and (c) water surface turbulence or high water velocity causing a broken surface.

Values of preference (D) were calculated from the formula of Jacobs (1974):

$$D = \frac{r-p}{r+p-2rp}$$

where r = proportion of resource use by the trout and p = proportion of resource available in the environment.

Results

Movements of Resident Brown Trout

A total of 19 trout were marked in the study area until September 1985. During October and November, no unmarked fish were captured, suggesting that 100% of the resident population was marked. This corresponds to a population density of 35 resident brown trout/ha. Six individuals were captured only once, while a total of 41 recaptures were made, varying individually in the range one to six recaptures.

In 1986, five of the trout marked in 1985 were recaptured, implying a population reduction during winter of 74%, i.e. resident trout which had either died or emigrated downstream. One of the trout had moved 900 m downstream, another 129 m upstream, while the other three were recaptured within 3–24 m of the last observation site in 1985. Six new resident trout were captured and marked, so the population of ressident trout was 11 prior to introduction of nonresidents. This corresponds to a population density of 20 resident brown trout/ha. Of the 1986 resident population, one individual was captured once and a total of 34 recaptures were made in the range 1–10 recaptures of individuals. Of these recaptures, 26 occurred after the introduction of nonresident trout.

The sparse resident brown trout population displayed some variation in movement distances at low population density (Fig. 2), but generally the movements were limited (Fig. 3). Thirty-five percent of the observed movements were less than ±18 m, 61% were less than ±50 m, while 20% had moved more than ±100 m. A hypothesis of normality was rejected ($P < 0.01$, Shapiro–Wilkes) due to leptokurtosis, i.e. more

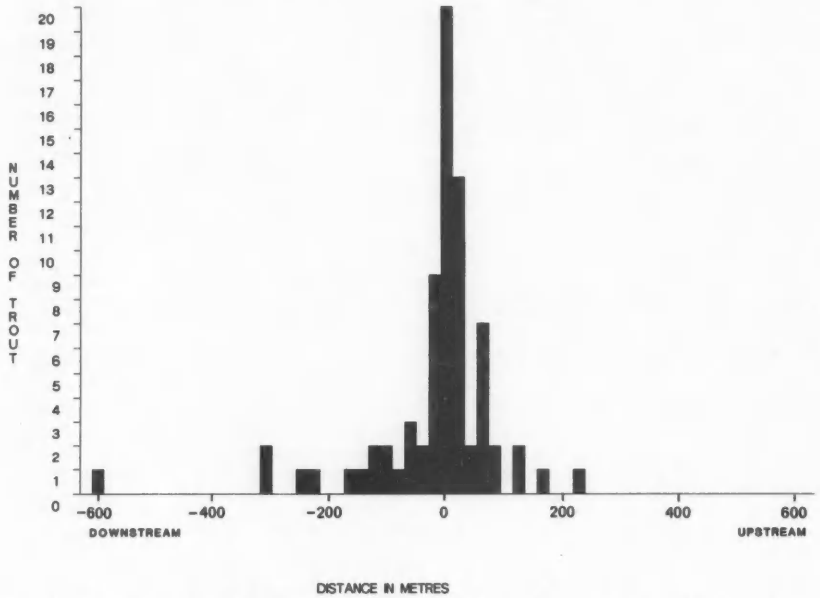

FIG. 2. Movements of resident brown trout in Hengselva. Each block represents a distance of 20 m.

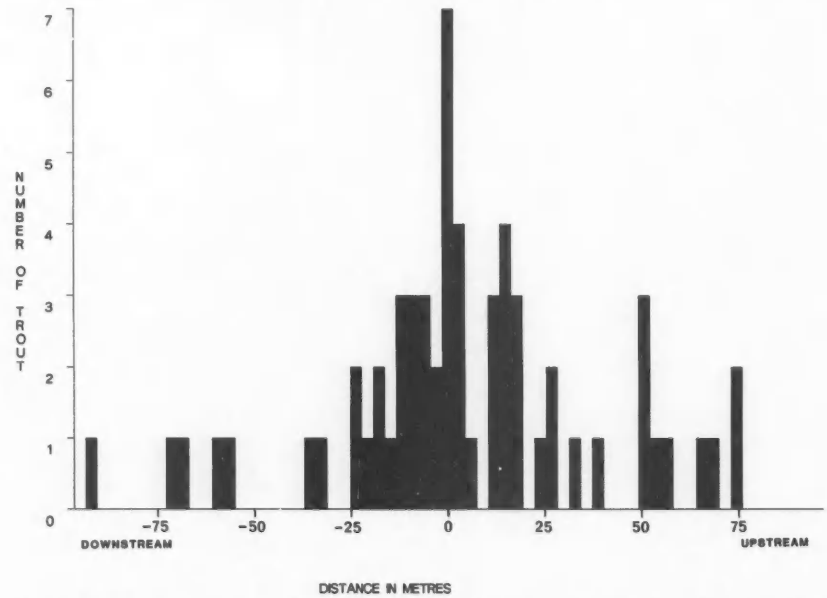

FIG. 3. Local movements (i.e. within ±100 m) of resident brown trout in Hengselva. Each block represents a distance of 3 m.

observations near the mean and the tails, with fewer observations in the intermediate regions relative to a normal distribution with the same mean and variance (g_2 = 1.5454 of the moment statistics; Sokal and Rohlf 1969). The curve was also slightly skewed in the downstream direction (g_1 = −0.9964 of the moment statistics). This was reflected in the mean moving distance, which was −18.7 m (SE = 16.9, n = 48), compared with a median of 0 m. If one long downstream migration of −588 m was deleted, mean moving distance was −6.7 m (SE = 12.1, n = 47). On average,

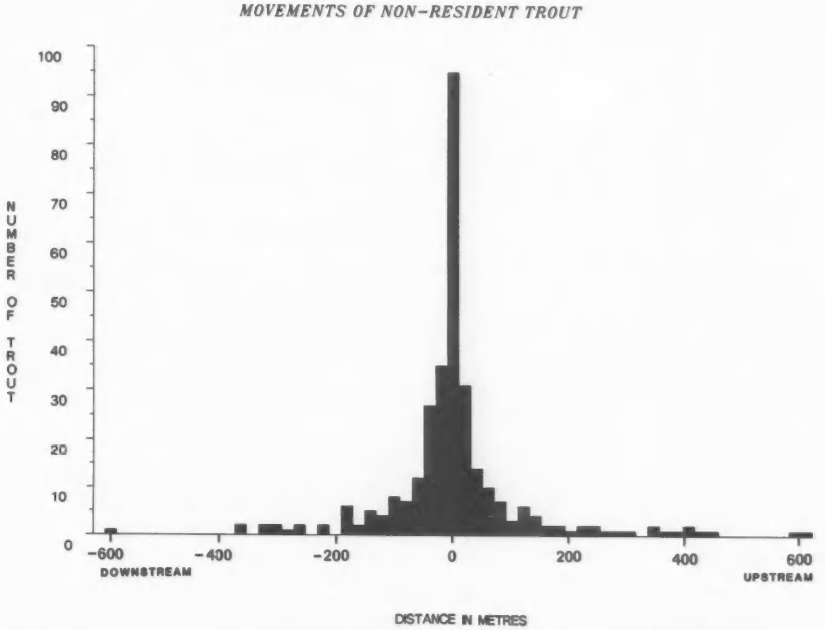

FIG. 4. Movements of nonresident brown trout in Hengselva. Each block represents a distance of 20 m.

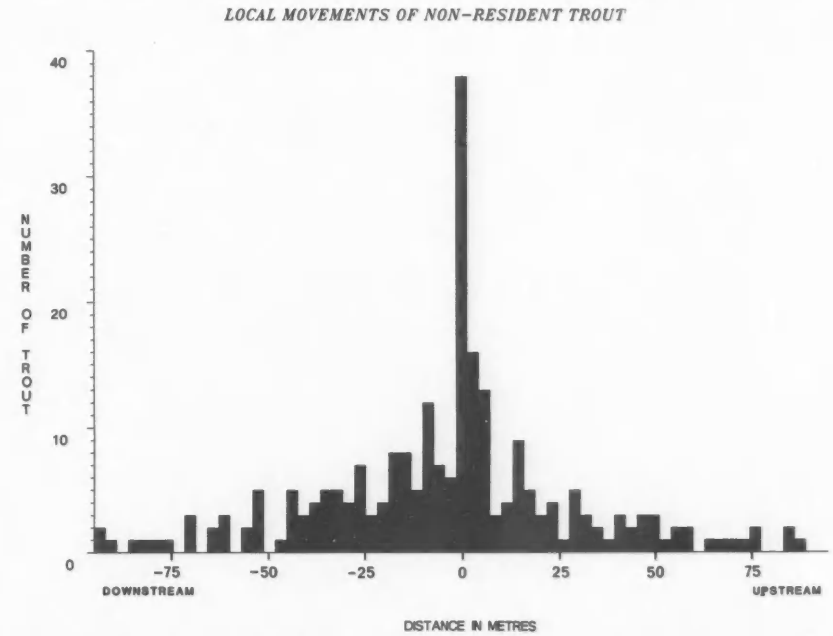

FIG. 5. Local movements (i.e. within ±100 m) of nonresident brown trout in Hengselva. Each block represents a distance of 3 m.

20.7 d elapsed between each recapture of resident trout.

No evidence was found of a changed movement pattern of the residents after introduction of nonresident wild trout, i.e. at low and high population densities ($P > F = 0.2376$, t-test), but the number of observations after introduction was small ($n = 26$).

Movements of Nonresident Brown Trout

The introduction of 130 nonresident trout raised the population density to 262 trout/ha from a previous level of 20 trout/

ha. Of the nonresident trout introduced, 29 individuals were not recaptured, while in all, 311 recaptures were made, varying from one to seven for individuals.

The movements of the nonresident trout within the study area at high population density followed a similar pattern to the resident trout (Fig. 4 and 5): 44% of the movements were less than ±18 m, 66% were less than ±50 m, and 23% were more than ±100 m. The curve was slightly skewed in the upstream direction ($g_1 = 0.4362$ of the moment statistics; Sokal and Rohlf 1969). Mean moving distance was 2.7 m (SE = 7.1, $n = 311$), while the median was 0 m. A hypothesis of normality was rejected ($P < 0.01$, Shapiro–Wilkes), again due to leptokurtosis, i.e. some rather long migrations and many movements near the mean ($g_2 = 4.4016$ of the moment statistics). The average number of days between each recapture was 23.6.

The movement pattern of nonresident brown trout at high population density did not deviate significantly from that of the pooled data for movements of resident trout ($P > F = 0.1853$, t-test). No changes in the movement pattern were found during the spawning season, presumably because sufficient spawning grounds were found within the study area. Several redds were observed.

Habitat Use by Resident and Nonresident Brown Trout

The introduction of nonresidents did not affect the habitat selected by resident trout with regard to depth and water velocity ($P < 0.01$, G-test), while for substrate and cover the comparison was just above the level of significance ($P = 0.07$ and $P = 0.08$). However, a closer examination of the original data suggested that the higher G-values of substrate and cover were not caused by any consistent changes, but rather were due to random errors because of the low number of observations of resident trout at high population densities ($n = 26$).

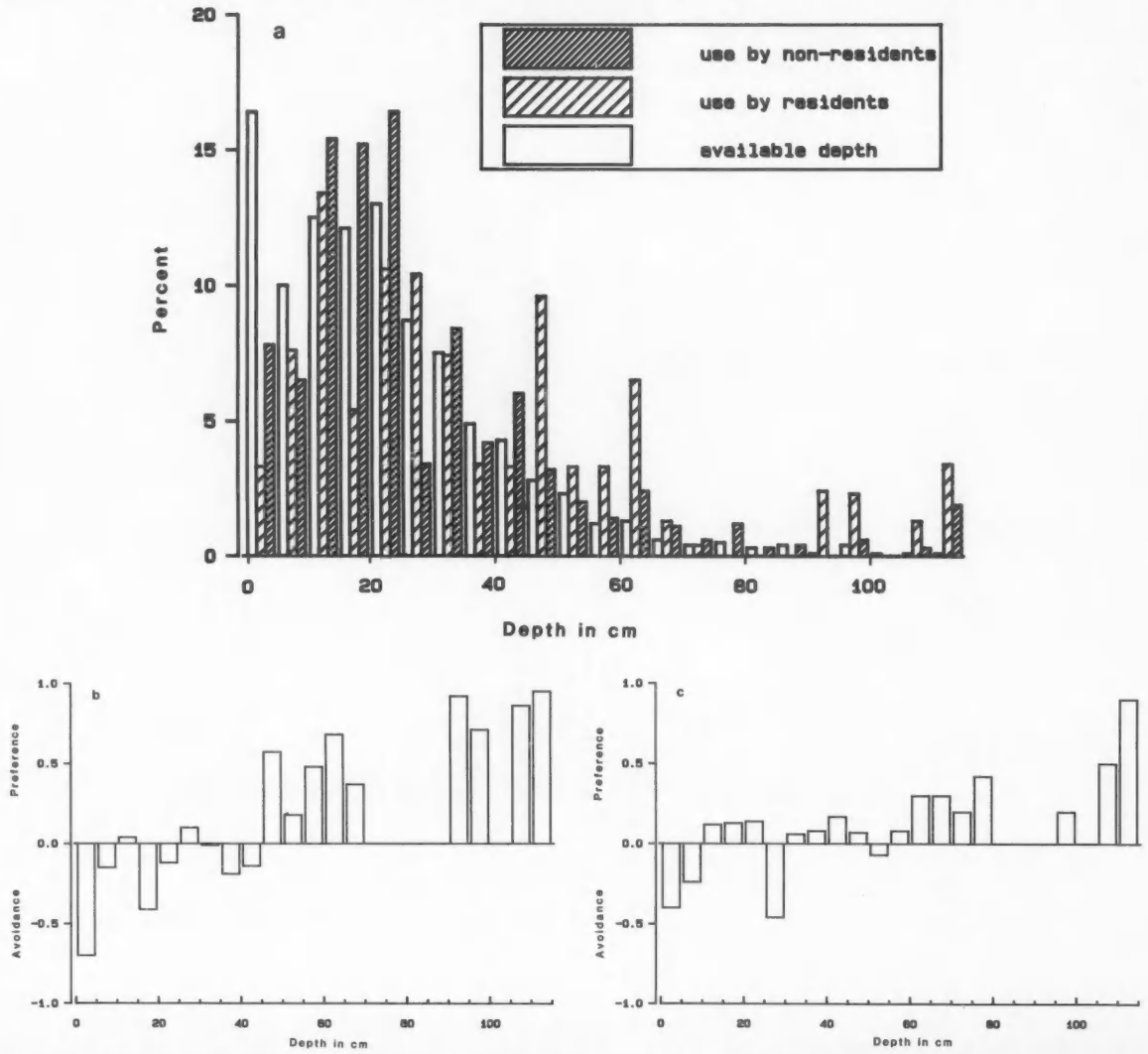

FIG. 6. (a) Depth availability and use, and preference (+) or avoidance (−) by (b) resident brown trout at low population density and (c) nonresident brown trout at high population density in Hengselva. Each block represents a depth interval of 5 cm.

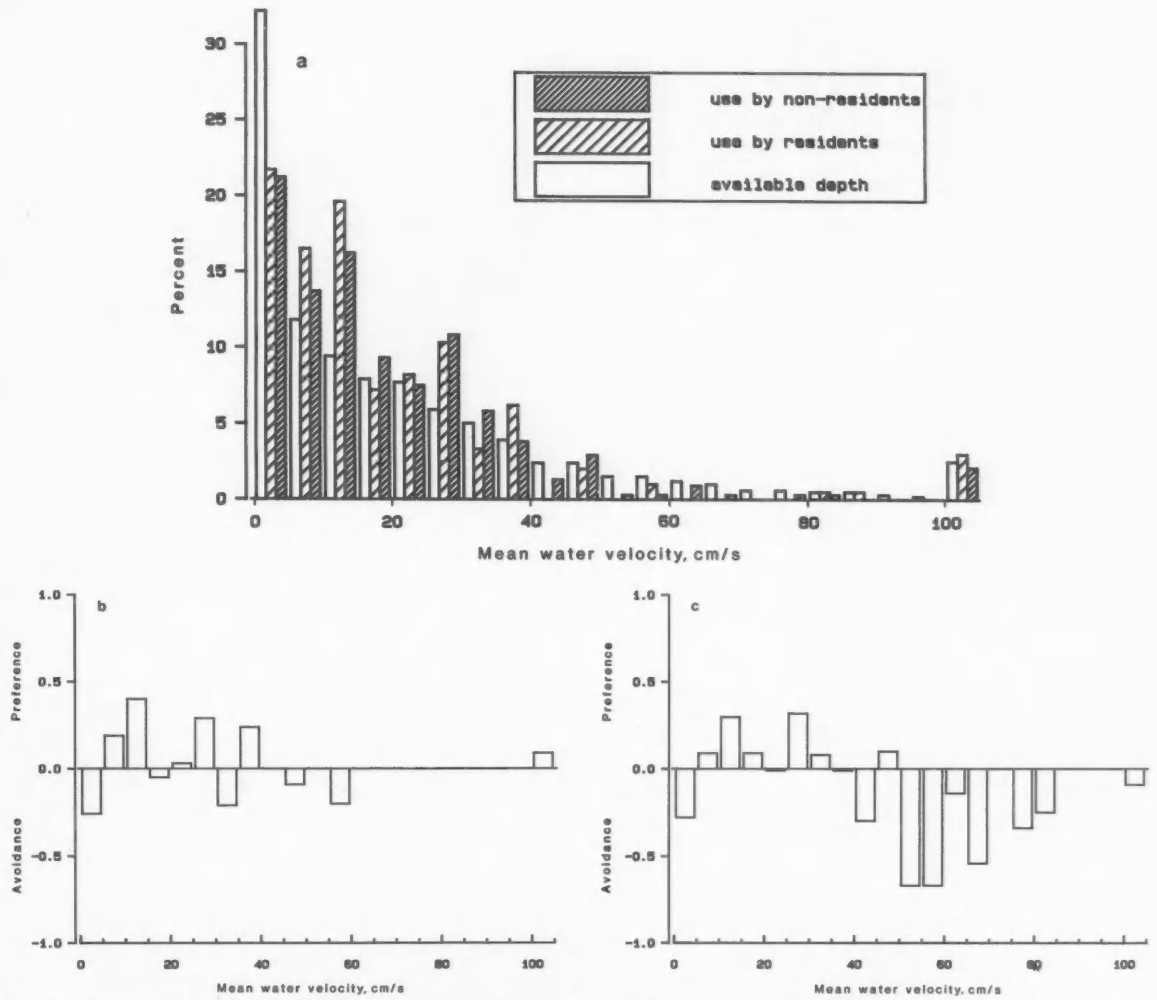

FIG. 7. (a) Mean water velocity, availability, and use, and preference (+) or avoidance (−) by (b) resident brown trout at low population density and (c) nonresident brown trout at high population density in Hengselva. Each block represents a velocity interval of 5 cm/s.

Habitat use by the resident trout at low density before and nonresident trout at high density after introduction is shown in Fig. 6–9. The resident trout were observed in significantly deeper stream areas with more cover and coarser substrate compared with the habitats chosen by the introduced nonresidents ($P < 0.01$, G-tests). However, the changes in substrate and cover preferences had lower consistencies than depth. As to the variables surface and mean velocities, no changes in preferences of residents at low compared with nonresidents at high population density could be discerned. At both high and low population densities, there was a distinct tendency for the larger trout to prefer the deeper pool areas. At low densities, mean size of trout in pools was 27.3 cm (SE = 0.7, n = 60) compared with the significantly smaller fish of 23.1 cm (SE = 0.1, n = 14) ($P < 0.01$, t-test) observed in other habitat types. Similarly, mean size of brown trout observed in pools at high population densities was 19.9 cm (SE = 0.5, n = 140) compared with 16.3 (SE = 0.2, n = 197) in other stream areas.

Habitat Preferences of Resident and Nonresident Brown Trout

Compared with total available habitat, both resident trout at low density and nonresident trout at high density displayed significant habitat preferences for all the measured variables ($P < 0.01$, G-tests), except for surface velocities for resident trout (Fig. 6–9). The trout clearly preferred the deeper stream areas, i.e. water >50 cm deep, and avoided high water velocities and areas with smooth bedrock substrate. A preference for cover was also observed.

Homing

Of 130 displaced trout, only two were recaptured during control fishing at their original "home" section. Both individuals were originally captured at the lowermost site 3.3 km downstream of the study area, but were both recaptured at the upstream end of the uppermost site, approximately 1 km down-

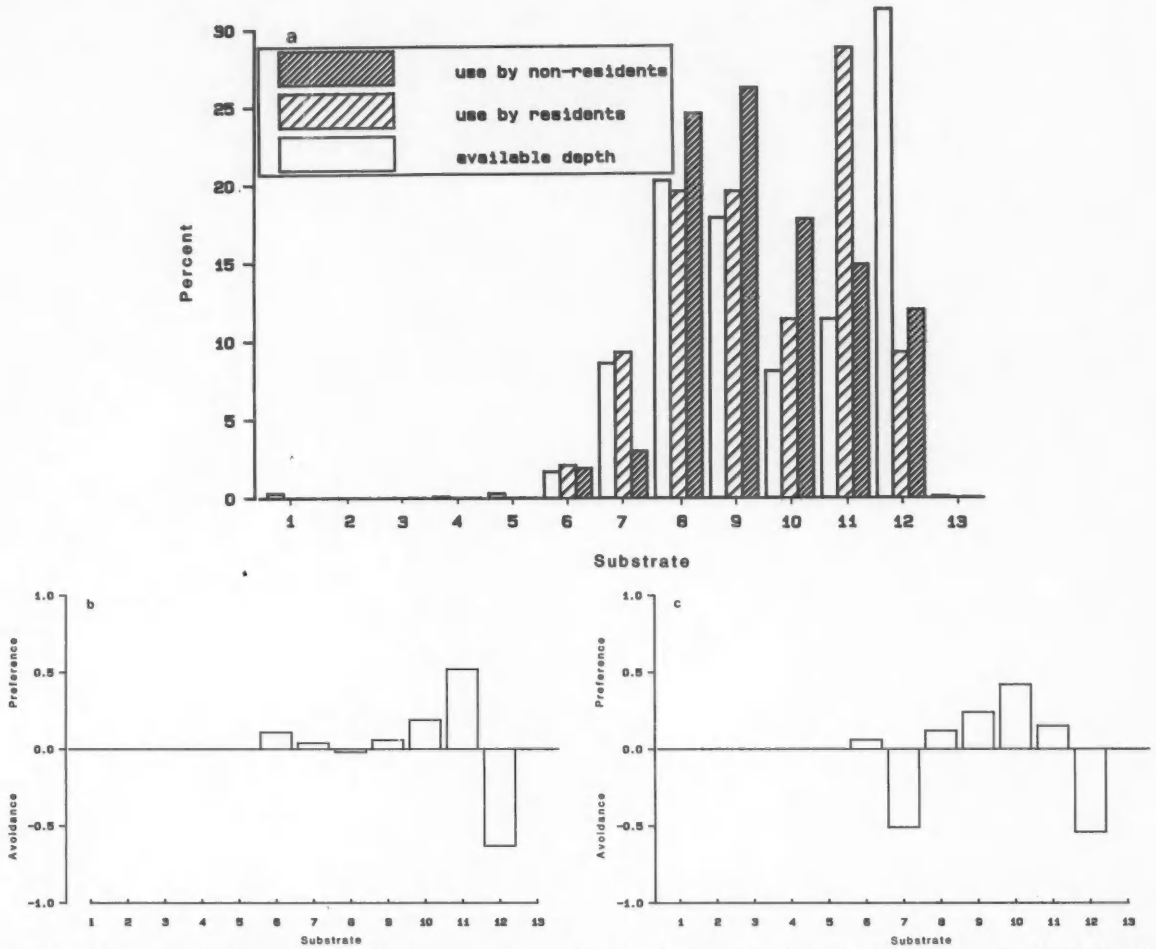

FIG. 8. (a) Substrate availability and use, and preference (+) or avoidance (−) by (b) resident brown trout at low population density and (c) nonresident brown trout at high population density in Hengselva.

stream of the limit of the study area. One was caught 1 mo after its displacement and the other 4 mo after displacement.

Discussion

Studies of stream-dwelling trout movements have in general indicated populations to be spatially stable, and movements have been reported within a restricted range from some hundred metres to a few kilometres (Shetter 1936; Shuck 1945; Allen 1951; Miller 1957). Individuals apparently tend to remain in a home range (Stefanich 1952; Holton 1953; Newman 1956; Bohlin 1977; Bachman 1984). Hatchery-reared trout released into flowing waters apparently also exhibit limited movement (Cresswell 1981).

The present study recorded short distance movements of brown trout equal to or greater than 3 m for 2 yr and suggests also that shorter movements for adult brown trout in streams are in general limited. Considerable local movements occurred mainly within a range of ±50 m.

From general population dynamics theory on the effect of territoriality and hierarchy on movements, a positive relationship between movement and population density would be expected (Gerking 1959). However, no effect of population density on movements of trout could be discerned in Hengselva. The movement pattern remained similar for resident trout at both low and high densities and also between resident and nonresident trout. Mense (1975) found no change in movement pattern for brown trout with fork lengths >15 cm when the population decreased by 50% from one year to another. He concluded that if a positive relationship between movement and population density exists, it exists at densities exceeding 209 trout/ha. In the present study, the 1986 high population density was 262 trout/ha, while the 1985 low density was 35 trout/ha.

A model of territoriality and hierarchy would also predict the dominant individuals to select home ranges in habitats affording maximum potential energy profit (Bachman 1984; Fausch 1984). Analysis of the present data indicated that brown trout inhabiting pools, i.e. deeper and more slow-flowing habitats, were on average of larger size than trout occupying other types of stream areas, both at high and low population densities. Trout inhabiting pools were therefore presumably dominant individuals. Jenkins (1969) and Bachman (1984) reported larger indi-

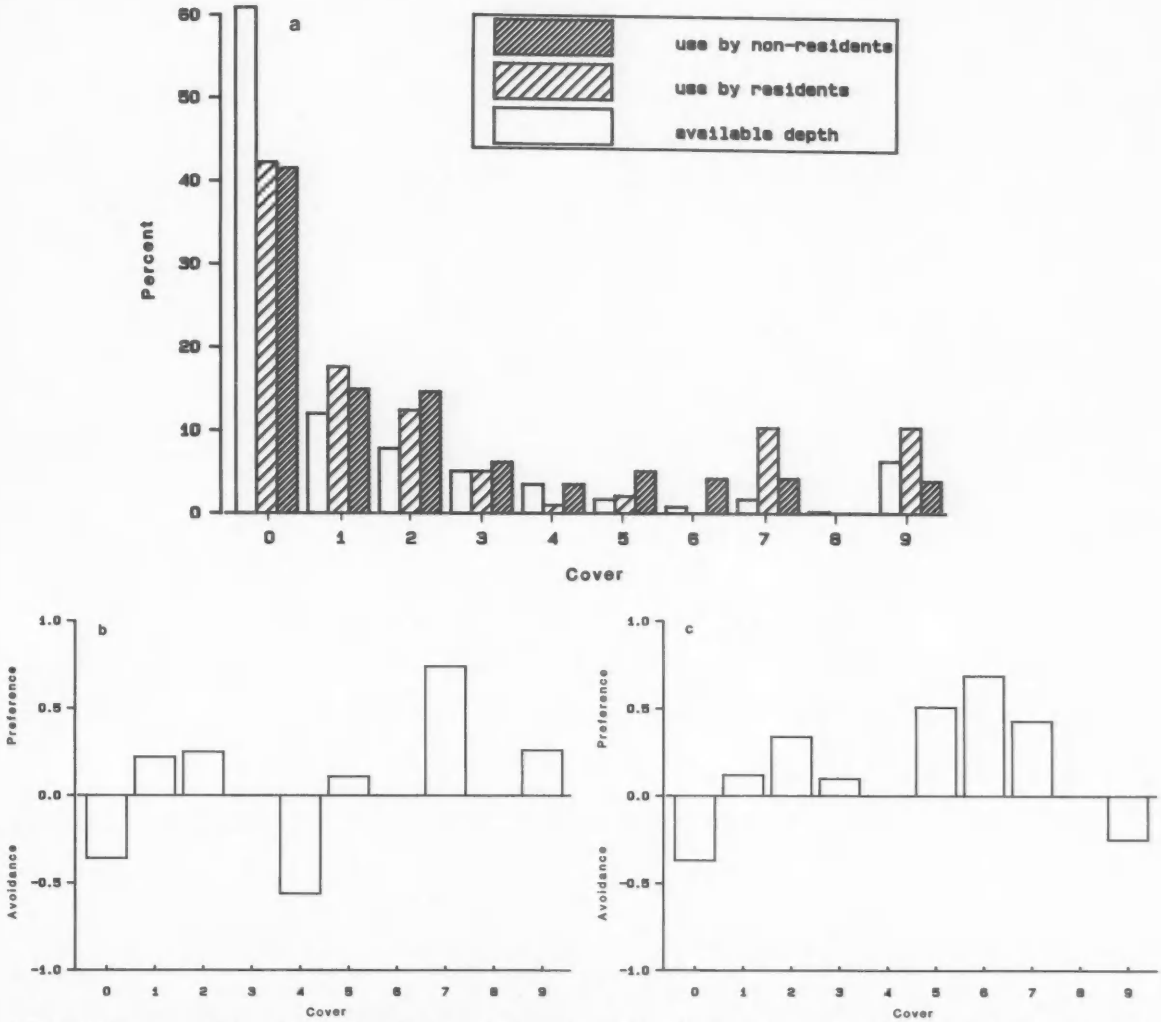

FIG. 9. (a) Cover availability and use, and preference (+) or avoidance (−) by (b) resident brown trout at low population density and (c) nonresident brown trout at high population density in Hengselva.

viduals to be in general dominant in brown trout hierarchies. In Hengselva the brown trout had a negative preference for shallow habitats with higher water velocities. The fish occupying such habitats were of smaller size. It therefore seems reasonable to assume that these fish were lower-ranking individuals excluded from more profitable stream areas by intraspecific competition from larger, dominant fish.

The introduced nonresident trout occupied habitats different from the residents. Habitats with less depth, less coarse substrate, and less cover, i.e. presumably more marginal habitats, were used to a larger degree by the nonresidents. This was probably a reflection of the increased intraspecific competition. Also, the introduced trout used suboptimal habitats instead of moving out of the area. The resident trout were on average larger than the introduced trout and therefore presumably dominant (Bachman 1984). Similarly, Jenkins (1969) observed that prior residence and fish size were important in dominance hierarchies among brown trout. In accordance with this, the resident, and larger, trout in Hengselva were found in significantly deeper stream areas with coarse substrate and a relatively high degree of cover, i.e. presumably habitats affording less energy expenditure and more protection compared with the nonresidents introduced.

Few studies on true brown trout habitat preference exist. Several studies of habitats actually occupied by brown trout have indicated depth (Shuck 1945; Lewis 1969; Karlstrøm 1977; Bohlin 1977; Wesche 1980; Shirvell and Dungey 1983; Cunjak and Power 1986), velocity (Shirvell and Dungey 1983; Bachman 1984; Hermansen and Krog 1984), substrate (Bohlin 1977; Karlstrøm 1977), and cover (Lewis 1969; Hunt 1977; DeVore and White 1978; Fausch and White 1981; Hermansen and Krog 1984; Cunjak and Power 1986; Wesche et al. 1987) to be important for brown trout, but different studies rank the importance of habitat variables differently. However, results from studies which have only measured habitats occupied by brown trout without quantifying total available habitat may be biased. (Shir-

vell and Dungey (1983) quantified available habitat in one of six study sites.) This may be one reason why ranking of habitat variables varies between studies in different streams.

As could be predicted from a model of optimum potential net energy gain (Fausch 1984), both resident and the nonresident trout in Hengselva avoided stream areas with high water velocities and smooth bottom, i.e. where low water velocity microniches were scarce (Bachman 1984). Smooth bedrock substrate was in general avoided. The most important habitat variable in Hengselva, i.e. for which both resident and nonresident trout showed the strongest preferences, was stream depth. This does not, however, necessarily imply that depth is the more important habitat variable for brown trout in streams. It is a reflection of the fact that in Hengselva the availability of deeper stream areas was restricted, and there was consequently a high preference for the limited deep areas available.

The degree to which trout were disturbed by sampling is difficult to assess. Brown trout are considered a wary species and even "point" electrofishing would probably disturb them. Although fish could be spotted in the stream due to the clear water, and all fish habitat observations were referred to the first point of observation (Hearn and Kynard 1986), they may still have been observed or captured at locations to which they had moved while attempting to avoid the disturbance. The measured habitat preferences may therefore be more representative of "refuge" or resting habitat than of daytime feeding locations.

Several studies have reported a homing tendency of brown trout after the spawning run (Shuck 1945; Stuart 1957; Solomon and Templeton 1976), but few studies of homing without any connection to spawning migrations exist. A homing tendency of displaced wild cutthroat trout (*Salmo clarki*) was reported by Miller (1954). Little homing tendency was observed in the present study. The displaced fish redistributed along the study area according to habitat conditions, but the movement pattern and downstream control fishing indicated minimal homing.

Acknowledgements

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Predation by Jellyfish on Larval Fish: An Experimental Evaluation Employing in situ Enclosures[1]

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de Lafontaine, Y., and W. C. Leggett. 1988. Predation by jellyfish on larval fish: an experimental evaluation employing in situ enclosures. Can. J. Fish. Aquat. Sci. 45: 1173–1190.

Large in situ enclosures were used to investigate the mortality of yolk-sac larval capelin (*Mallotus villosus*) by four species of jellyfish predators. Daily instantaneous larval mortality increased linearly with predator density but was independent of initial larval density, indicating a linear functional response of the four jellyfish species. The addition of alternative prey did not modify the linearity of the functional response and did not significantly reduce or increase larval mortality imposed by two jellyfish species. The evidence of a linear response in large enclosures contrasts with the curvilinear (Type II) response previously reported in studies conducted in small laboratory containers. Larval mortality was strongly dependent on predator size within jellyfish species and increased with initial larval size. Larval capelin appeared more susceptible to predation at the time of transition from endogenous to exogenous feeding. Daily larval mortality rates due to predation varied between 0.015 and 1.58 depending on jellyfish species and appeared more strongly determined by body mass than differences in feeding appendages of jellyfish. Our results indicate that reduced larval mortality to jellyfish predation is achievable only by timing larval release in water mass where jellyfish numbers are naturally reduced. We estimated that macro invertebrate predation has the potential of being the primary regulator of larval survival and we hypothesized that large intra- and interannual variation in larval survival may be due to the variability in the emergence timing of capelin larvae relative to the growth trajectory of jellyfish.

De larges enclos in situ ont été utilisés pour étudier la mortalité des larves de capelan (*Mallotus villosus*) avec sac vitellin en présence de quatre espèces de méduses pélagiques. Le taux journalier instantané de mortalité larvaire induite a augmenté linéairement avec la densité des prédateurs mais était indépendante de la densité initiale des larves suggérant une réponse fonctionnelle linéaire chez les quatre espèces de méduses. L'addition de proies alternatives n'a pas modifié la linéarité de la réponse fonctionelle et n'a pas significativement réduit ou augmenté la mortalité larvaire imposée par deux espèces de méduses. La mise en évidence d'une réponse fonctionnelle linéaire chez les quatre espèces de méduses dans de larges enclos contraste avec la réponse curvilinéaire (type II) préalablement observée lors d'études avec de petits contenants expérimentaux. La mortalité larvaire était fortement dépendante de la taille des prédateurs d'une même espèce et a augmenté avec la taille initiale des larves. Les larves de capelan apparaissent plus susceptibles à la prédation au moment de la transition d'un mode de nutrition endogène à exogène. Les taux de mortalité larvaire due à la prédation ont varié entre 0,015 et 1,58 selon les espèces de méduses et apparaissent plus fortement reliés à la masse corporelle qu'à des différences des appendices nutritionnels des méduses. Nos résultats révèlent que la réduction de la mortalité larvaire due à la prédation par les méduses n'est réalisable seulement lorsque la libération des larves a lieu à l'intérieur de masses d'eau où l'abondance des méduses est naturellement réduite. Nous estimons que la prédation par les organismes macroinvertébrés peut être le régulateur primaire de la survie des larves et nous émettons l'hypothèse que la variation intra- et interannuelle chez la survie larvaire soit liée à la variabilité entre le temps d'émergence des larves de capelan et la trajectoire de croissance des méduses.

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The paucity of solid evidence in support of the starvation hypothesis, in spite of over 60 yr of study (Leggett 1986), has led to the repeated suggestion that predation may be the major source of death for fish eggs and larvae (Murphy 1961; Cushing and Harris 1973; Cushing 1974, 1983; Ware 1975, 1980; Johannes 1978; Hunter 1981, 1984; Sissenwine 1984). Given their numerical abundance and their widespread distribution, carnivorous macrozooplankters are frequently considered important predators of the early larval stages of fishes (Harding and Talbot 1973; Möller 1980, 1984; Brewer et al. 1984; Purcell 1985).

Brewer et al. (1984) considered that field estimates of "apparent" predation on larval fish, mostly based upon gut content analyses of predators, ultimately require a more direct and more controlled quantitative determination of predation rates. Laboratory studies of predation have contributed primarily to the behavioral and quantitative description of the func-

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tional response between larval fish and their potential predators (Hunter 1984; Purcell 1985; de Lafontaine and Leggett 1987b). Studies on several species of macrozooplankton have revealed that jellyfish yielded the highest predation rates on larval fish (Bailey and Batty 1983, 1984; Bailey 1984; Purcell 1985). Extrapolation of the results of laboratory studies to field conditions must be approached with caution because predation rates imposed on larval fish by jellyfish are strongly dependent on the size of the experimental container employed (de Lafontaine and Leggett 1987b). Large in situ enclosures can yield results at a scale more relevant to the field conditions (de Lafontaine and Leggett 1987a, 1987b) and permit larval densities more "natural" than those generated in using small containers.

Several other variables (the presence of alternative prey, predator density, size of predators and prey) are also known to be important determinants of the rate and form of the predator–prey relationship in general (Murdoch and Oaten 1975; Hassell 1978; Taylor 1984; Houck and Strauss 1985). The addition of alternative prey was shown to decrease the predation rates on larval prey for some predators (Lillelund and Lasker 1971; Bailey and Yen 1983; Kuhlmann 1977; von Westernhagen and Rosenthal 1976) but this effect has not been quantified for jellyfish predators. Although it is generally hypothesized that predation mortality during the early life stages of fish should be proportional to predator numbers (Hempel 1963; Jones 1973; Ware 1975; Cushing and Harris 1973), the effect of predator density on the individual predation rates of jellyfish is still unknown. To date, the functional response of jellyfish fed on larval fish has been quantified for *Aurelia aurita* only and comparison between different jellyfish species is lacking.

The lack of information on the relative importance of these factors in determining the relationship between predation rate and larval density has precluded development of a general understanding of predator–prey interactions as they apply to larval fishes. The present study examined the effects of larval fish density, predator density, and presence of alternative prey on larval fish mortality rates imposed by jellyfish predators. Specifically the following hypotheses were evaluated: (1) larval mortality due to predation should be directly proportional to the abundance of jellyfish predators; (2) larval fish mortality due to predation should be inversely related to the initial larval density; and (3) larval mortality due to predation should be significantly reduced in the presence of alternative food available for predators.

Materials and Methods

Enclosure Studies

Predation experiments were conducted during the summers of 1982, 1983, and 1984 using 3.2-m^3 in situ enclosures moored in Bryant's Cove, Newfoundland. Cylindrical Dacron enclosures with a conical bottom constructed of 53-μm Nitex were employed in all but two series of experiments conducted in 1982. In these two experiments, full Dacron enclosures were used. All enclosures were allowed to fill by diffusion of water through the porous walls. Full details of construction, physical and biological characteristics, and procedures for deployment and retrieval of the enclosures are described elsewhere (de Lafontaine and Leggett 1987a).

Yolk-sac larvae of capelin (*Mallotus villosus*) were used as prey. All larvae were collected from the spawning beach at Bryant's Cove immediately prior to stocking. To collect larvae, beach gravel containing recently hatched larvae was mixed with seawater in a bucket to release the larvae. Actively swimming larvae were captured with a large-diameter pipette, counted, and placed in 500-mL glass jars filled with filtered seawater. The closed jars were suspended in the enclosures for approximately 1 h to allow temperature acclimation prior to the release of larvae. A sample of 100 larvae preserved in 10% buffered formalin seawater immediately after capture was used to determine the physical condition of the larvae initially stocked for each experiment in 1983 and 1984. The vertical distribution and the diel migration of capelin larvae in the enclosures have been described in de Lafontaine and Leggett (1987a).

The jellyfish species used as predators in the experiments were *Catablema vesicarium* (Hydrozoa, Anthomedusae), *Staurophora mertensi* (Hydrozoa, Leptomedusae), *Cyanea capillata* (Scyphozoa), and *Aurelia aurita* (Scyphozoa). These species have previously been shown to capture and ingest fish larvae (Fraser 1969; Purcell 1985). Jellyfish were individually trapped in glass jars by SCUBA divers and transferred immediately to the enclosures. The number of jellyfish added to each enclosure was constant within each experiment, but varied among experiments (two to five per enclosure), depending on their availability. The resulting predator densities (0.6–1.5·m^{-3}) approximated the average in situ densities of predators in this area (range = 0.1–10·m^{-3}; Frank and Leggett 1982).

Alternative prey for the predators consisted of wild zooplankton collected at depths ranging from 0 to 5 m with a large-volume plankton pump (Taggart and Leggett 1984) operated at a monitored flowrate of 250–300 L·min^{-1}. To avoid adding large predators during pumping, the pump discharge was passed through a 1000-μm screen suspended inside the enclosures. During each food addition, three samples of volumes equivalent to those added to the enclosures were taken to determine the initial abundance and size distribution of the zooplankton added. The number of capelin larvae accidently added to the enclosures during pumping was also estimated from these samples. Larval densities were subsequently corrected for this bias. These additions proved to be minimal (maximum of 35 larvae per enclosure).

Experiments were initiated shortly before dusk. Some experiments lasted up to 96 h, but the majority (89%) had a duration of 36–46 h (mode 39 h). At the end of each experiment, all enclosures were lifted, and their contents (fish larvae, predators, alternative food) were preserved in 10% buffered formalin seawater. Because temperature, salinity, and oxygen levels inside the enclosures were similar to the surrounding waters (de Lafontaine and Leggett 1987a), temperature and salinity recorded at a depth of 4.5 m with a Aanderaa currentmeter moored 30 m from the enclosures were used to monitor the conditions in the enclosures.

The effect of larval density on predation mortality was determined for the four jellyfish species listed above. The experimental design employed a maximum of 10 enclosures to simultaneously test a wide range of unreplicated larval densities. During each trial, one enclosure, stocked at a preselected larval density but lacking predators, served as control. These experiments were replicated in time. Preliminary experiments using replicated larval densities were conducted in 1982 to assess the coefficients of variation in predation mortality at a given prey density. In these experiments, three enclosures each containing predators and one control enclosure containing larvae but lacking predators were simultaneously deployed.

Experiments involving different larval densities were therefore conducted on different dates. The initial density of capelin larvae in all experiments was varied between 12 and 780 larvae·m^{-3}. This spans the natural range of densities of newly emergent capelin larvae reported for Bryant's Cove (Frank and Leggett 1981, 1982) and for the estuary and Gulf of St. Lawrence (de Lafontaine et al. 1984; Fortier and Leggett 1985).

The effect of predator density on larval mortality was evaluated in one experiment by stocking a fixed number of larvae (300) into nine enclosures containing 1, 4, 7, or 10 *Aurelia aurita*. Replicates were used for enclosures containing 1, 4, and 10 predators, and only one enclosure was used for the density involving 7 predators.

The effect of alternative food on the larval mortality imposed by jellyfish predators was investigated in two different ways. The effect of larval density on mortality rates in the presence of relatively constant alternative food densities was first evaluated. In each experiment, two control enclosures containing larvae and alternative food but lacking predators were employed to evaluate possible interaction effects between zooplankton and larvae. Three series of such experiments employing *Staurophora mertensi* and two using *Aurelia aurita* as predators were performed. In these experiments the quantity of zooplankton added to each enclosure was regulated by pumping equal volumes of water into each enclosure. However, because of small-scale patchiness, some between-enclosure variation (coefficient of variation (CV) 1–23%) in initial zooplankton density occurred. The density of stocked zooplankton also varied between series of experiments because of temporal variation in in situ zooplankton abundance. A second series of experiments were conducted to evaluate the effect of alternative food density on predation rates. In these experiments, the number of capelin larvae (250) was fixed and the quantity of zooplankton added was varied by altering the volumes pumped by a ratio of 1:15. Five series of such experiments were completed, two using *Aurelia aurita* as predators and three using *Staurophora mertensi*.

In all experiments, treatments were randomly assigned to the enclosures.

Laboratory Analyses

The number of larvae recovered from each enclosure at the termination of the experiments was determined. Total length, body height behind the pectoral fins, and yolk-sac volume were measured for a maximum of 40 larvae selected at random. Yolk-sac volume was estimated using the spheroid equation (Laurence 1973):

$$\text{Yolk volume} = (3.14159 \cdot \text{yolk length} \cdot (\text{yolk depth}^2))/6.$$

Predator size (umbrella diameter) was measured and was corrected for shrinkage due to formalin preservation (Möller 1980; de Lafontaine and Leggett 1988). Jellyfish fresh weight was estimated from umbrella diameter–fresh weight relationships.

Initial and final counts and the size distribution (80–520 μm ESD) of zooplankton used as alternative prey were obtained with a Coulter model TA-II particle counter using a 2000-μm-aperture tube. Total abundance (number per litre) and total biomass (milligrams per litre, derived from Coulter counter volume estimates) were estimated for each zooplankton sample. The computed ratio of total biomass to total abundance provided an estimate of the average size (microns) of alternative food added to the enclosures in each experiment. Mean and standard deviation of water temperature during each experiment were computed from currentmeter data.

The instantaneous daily larval mortality rate due to predation (Z, per predator per day) was calculated as

$$(1) \quad Z = ((\ln N_i/N_f) - (\ln N_i/N_c))/T \cdot P$$

where N_i is the initial number of larvae stocked, N_c and N_f are the final number of larvae in the control enclosures and in the enclosures with predators, respectively, P is the number of predators added to the enclosures, and T is the duration of the experiment in days. This equation corrects for prey depletion during experiments in which ingested prey are not replaced (Royama 1971; Rogers 1972; Arditi 1982). Daily predation rates (number of larvae per predator per day) were calculated as

$$(2) \quad I = N_i \cdot (1 - \exp(-Z)).$$

All statistical tests were done on the Z values because we sought to identify the factors that affect predation mortality rates on larvae rather than to define the exact parameters of the functional response. This approach was chosen because (1) the exact parameterization of the functional response is statistically difficult and can yield biased interpretations in comparative studies (Arditi 1982; Livdahl and Stiven 1983; Houck and Strauss 1985; Williams and Juliano 1985) and (2) the variation of the two parameters of the functional response (attack rate and handling time) relative to different factors (predator size, prey size, abundance of alternative food, environmental conditions) is difficult to resolve given the mathematical form of the curve.

The relationship between Z and initial larval density was investigated using an analysis of variance blocked by time and by a regression analysis. The statistical significance of the effect of alternative food on larval mortality rates was evaluated by comparing results from experiments conducted in which alternative food was present and absent. The data from different experiments were pooled and standard regression residuals were computed and analyzed by F-test (ANOVA) and also by nonparametric Mann–Withney U-test as recommended for comparative analysis of predation rates (Houck and Strauss 1985).

Results

Larval Mortality due to Capture and Handling

Larval mortality due to capture and handling was assessed by placing 150–200 capelin larvae in each of 15–20 sealed glass jars filled with 500 mL of filtered seawater. The jars were then immersed in a land-based tank supplied with continuously running seawater pumped from Bryant's Cove. Two or three times each day for a period of 1 wk, one jar was randomly selected and dead and live larvae in the jar were separately preserved. This experiment was repeated three times (16–24 July 1983, 14–22 July 1984, and 21–29 July 1984). Cumulative mortality was consistently <5% during the first 96 h, the maximum duration of our experiments (Fig. 1A). The high mortality observed after 5 d in experiment II and at day 7 in experiment III is believed to have resulted from starvation. Larval mortality induced by capture and handling was therefore considered negligible.

The length of live capelin larvae held in jars without food increased by approximately 0.5 mm during the first 72 h and remained stable thereafter (Fig. 1B). This growth was accompanied by a corresponding reduction in yolk reserves which were virtually exhausted by 96 h (Fig. 1C). Similar length increases and yolk decreases were observed during larval residence in control and experimental enclosures. Larval

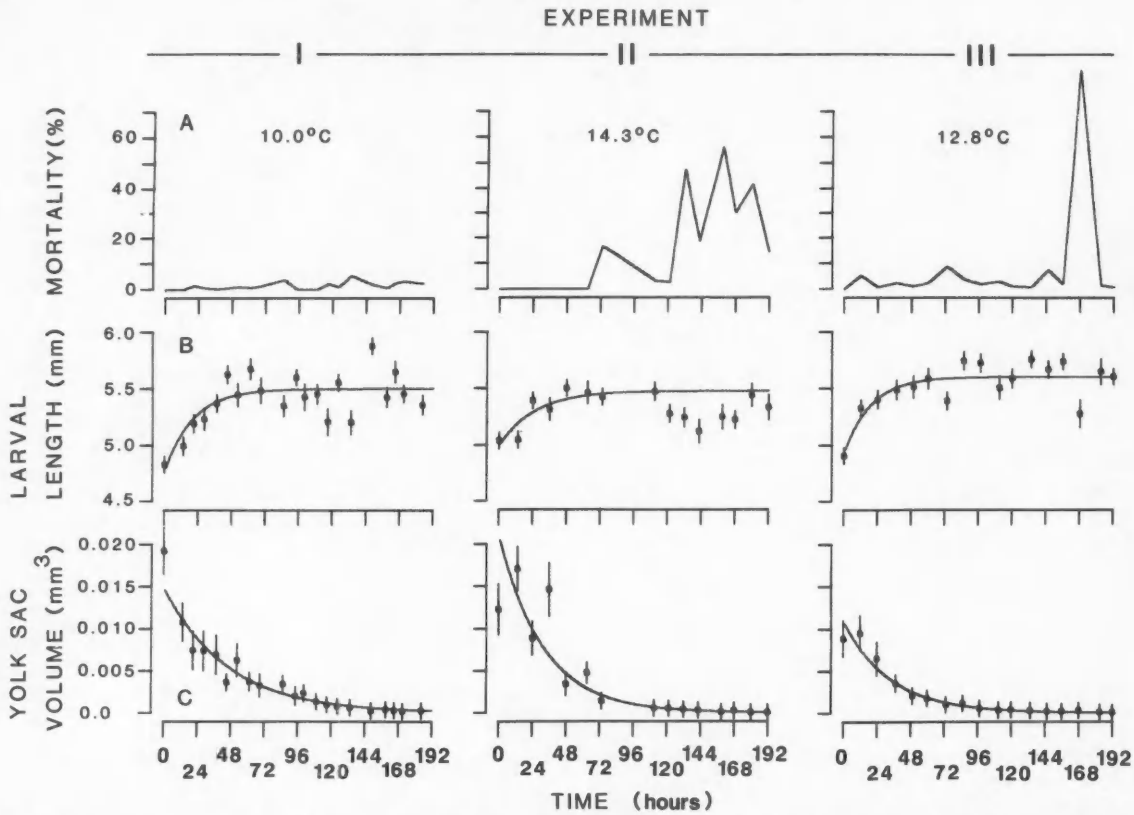

FIG. 1. Percent mortality, total length, and yolk-sac volume of capelin larvae held for 1 wk in glass jars without food during three different experiments. Relationships of larval length (TL) over time (t in hours) as follows: Exp. 1, $TL = 5.5 - 0.725 \cdot \exp(-0.051t)$, $r^2 = 0.83$; Exp. 2, $TL = 5.45 - 0.461 \cdot \exp(-0.044t)$, $r^2 = 0.80$; Exp. 3, $TL = 5.6 - 0.671 \cdot \exp(-0.052t)$, $r^2 = 0.82$. Relationships between yolk-sac volumes (YV) and time (t) as follows: Exp. 1, $YV = 0.0133 \cdot \exp(-0.021t)$, $r^2 = 0.95$; Exp. 2, $YV = 0.0117 \cdot \exp(-0.028t)$, $r^2 = 0.95$; Exp. 3, $YV = 0.0084 \cdot \exp(-0.024t)$, $r^2 = 0.96$. All regressions are significant ($p < 0.001$). Vertical bars represent standard errors of the mean.

condition index computed as the ratio of total length to body height (Ware et al. 1981; Neilson et al. 1986) differed significantly between live and dead larvae removed from the jar experiments during the first 120 h (Fig. 2A). (live: 32.36, SE = 3.13; dead: 20.09, SE = 6.07; $F = 1240.2$, $p < 0.0001$) but did not vary between experiments ($F = 1.04$, $p = 0.31$). The condition index allowed reliable identification of the proportion of dead and live larvae at the termination of each enclosure experiment.

Condition indices of larvae from both control and experimental enclosures (Fig. 2C) were significantly (Kolmogorov–Smirnov test, $p < 0.001$) higher than those of larvae initially stocked (Fig. 2B). This is due to larval growth during enclosure experiments. Comparison of the condition factor of larvae recovered from the enclosures with that of dead larvae from the jar experiments leads us to conclude that the majority (>90%) of larvae recovered from the enclosures were alive when experiments were terminated. The condition of larvae recovered from control and experimental enclosures did not differ significantly (Kolmogorov–Smirnov test, $p > 0.5$). We conclude that size-selective predation was not an important potential bias in our experiments.

Larval Recovery in Control Enclosures

Control enclosures lacking alternative food yielded better survival (96.3%, SE = 2.0) than did control enclosures with alternative food (91%, SE = 1.4, $F_{1,30} = 9.0$, $p = 0.0005$). The recovery rates of larvae from control enclosures which lacked alternative food were not correlated with initial larval density stocked ($r = 0.05$, $p = 0.82$) or with water temperature during the experiments ($r = 0.10$, $p = 0.63$).

Instantaneous larval mortality rates (Z) in control enclosures with alternative food were highest when the proportion of zooplankton in the >250-μm size classes was highest (Fig. 3, Exp. B,D,E, and F). A canonical discriminant analysis indicated that these four experiments formed a group distinct from the other experiments, with size spectra characterized by high abundance of particles <250 μm (Table 1). Mortality rates were positively correlated with the abundance of particles >518 μm ($r = 0.50$, $p = 0.026$) and with the average size of zooplankton ($r = 0.53$, $p = 0.016$). A stepwise multiple regression analysis was used to investigate the effect of initial density of larvae, average size of alternative food, abundance of particles (total and for each size class), temperature, and initial condition of larvae (from both larval condition index and yolk-

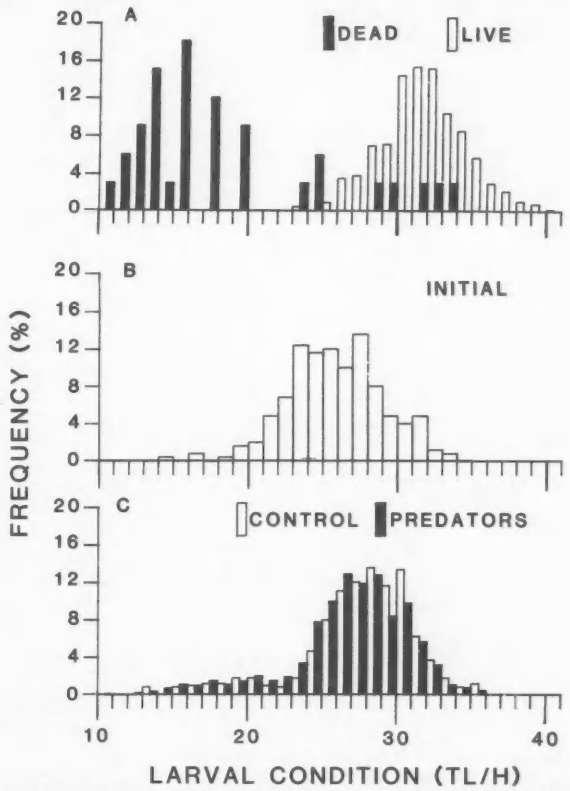

FIG. 2. Frequency distribution of larval condition index (total length/body height) for (A) dead and live larvae recovered from the jar experiments during the first 120 h, (B) larvae initially stocked to the enclosures, and (C) larvae recovered from control enclosures and from enclosures with jellyfish predators.

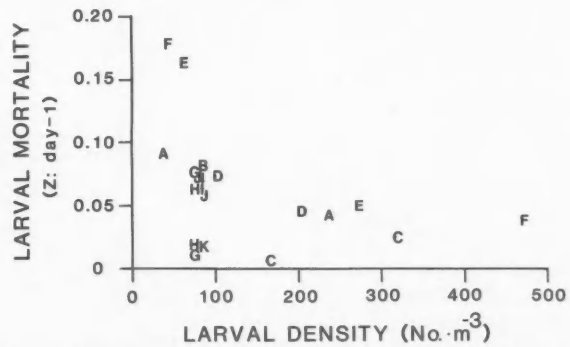

FIG. 3. Instantaneous mortality rate (Z) of larval capelin as a function of initial larval density in enclosures stocked with alternative food but lacking jellyfish predators. Letters correspond to experiments listed in Table 1.

sac volumes) on observed mortality rates of larvae in control enclosures. The average size of zooplankton (X_1) and the initial density of larvae (X_2) were the only variables selected. They explained 40.3 and 29.4%, respectively, of the overall variability in mortality rates ($Z = 0.2753 + 5.814X_1 - 0.044 \ln(X_2$; $r^2 = 0.70$, $p < 0.0001$). We conclude that the slightly lower (by about 5%) survival rates of larvae in enclosures with alternative food resulted from predation by larger elements of the plankton added as alternative prey for the jellyfish.

Effect of Jellyfish Density

Instantaneous larval mortality, uncorrected for the number of predators in the enclosures (Z, per day), was linearly related to the numbers of predators present in the enclosures (Fig. 4). The instantaneous larval mortality rate imposed per predator (Z, per predator per day) was independent of the predator number (ANOVA, $F_{3,5} = 0.19$, $p = 0.90$). This result justifies the use of equation 1 to correct Z for the number of predators present in the enclosure. The CV's (100·SD/mean) in mortality rate per predator were low, ranging from 11 to 24% (average 15%). Covariance analysis revealed no significant effect of mean predator size ($p = 0.98$) on the residual variability observed. As a result of the strong relationships between density and biomass of predators in the enclosures, total mortality (Z) was therefore strongly and linerly related to total umbrella area ($r^2 = 0.97$) and total biomass ($r^2 = 0.91$) of predators.

Effect of Larval Density

Larval mortality rates imposed by *Aurelia aurita* (Fig. 5) were consistently negatively, but never significantly, correlated with initial larval density in four different experiments (Table 2). The CV's in Z were low (17–41%) in the first three trials, and a high CV (81%) was observed in the fourth experiment due to two high Z values at intermediate larval densities (Fig. 5D).

Mortality rates and mean predator size were positively related in the first three experiments and negatively related in the last (Table 2). None of these relationships was significant ($p > 0.05$), however. Results from a stepwise multiple regression analysis indicated that larval mortality rates were independent of initial larval density and mean predator size. Larval mortality rates were therefore standardized for predator size using the following equation:

$$(3) \quad Zs_i = \frac{Z_i \cdot (\Sigma D_i / n)}{D_i}$$

where Zs and Z are the standardized and the nonstandardized mortality rates, D is mean size of predators in each enclosure, and n is the number of observations (enclosures) in each series of experiments. This allows removal of the residual variability in Z due to mean predator size for each enclosure and scaling (or standardizing of the magnitude of Z relative to the overall mean size of predators in one experiment. The relationships between standardized mortality rates and larval density were not improved and remained nonsignificant in the four experiments with *Aurelia aurita*.

The variances of mortality rates being homogeneous (F_{max} test = 8.5; $p > 0.05$), an ANOVA performed on larval mortality rates (Z) due to *Aurelia aurita* revealed a significant difference between experiments ($F_{3,25} = 10.15$, $p < 0.0001$). An a posteriori multiple mean test (Duncan test, SAS Institute Inc. 1982) indicated that significant differences in mortality rates occurred among the first three experiments, but that no difference existed between the first and fourth experiments. Variability in experimental conditions between experiments was thus more important than the effect of larval density on measured mortality rates due to predation. The increase in mortality rates

TABLE 1. Abundance, biomass, and average size of zooplankton used as alternative prey in predation experiments. Size spectrum letters correspond to experiments in Fig. 3. Values in parentheses indicate CV's (100·SD/mean).

Date	n	Abundance (no.·L^{-1})	Biomass (mg·L^{-1})	Average size: Volume (10^6 µm^3)	Average size: ESD (µm)	Size spectrum
1983						
17 July	3	19.614 (22.5)	0.1055 (20.6)	5.4099	217.8	A
19 July	3	24.387 (17.6)	0.3072 (27.4)	12.4347	287.4	B
28 July	3	133.541 (17.5)	0.4057 (26.7)	3.0024	179.0	C
2 August	3	13.464 (19.3)	0.1695 (18.5)	12.5957	288.7	D
6 August	2	30.753 (0.8)	0.4681 (8.5)	15.2270	307.5	E
8 August	3	6.988 (8.9)	0.0999 (18.1)	14.2249	300.6	F
1984						
13 July	3	275.740 (20.2)	0.6243 (10.8)	2.2949	163.6	G
23 July	3	176.342 (25.3)	0.7119 (8.5)	4.1544	199.5	H
25 July	2	121.307 (51.0)	0.6152 (51.0)	5.0715	213.2	I
27 July	4	201.148 (57.5)	0.6550 (69.8)	3.1008	180.9	J
31 July	4	147.329 (11.9)	0.7089 (9.3)	4.8367	209.8	K

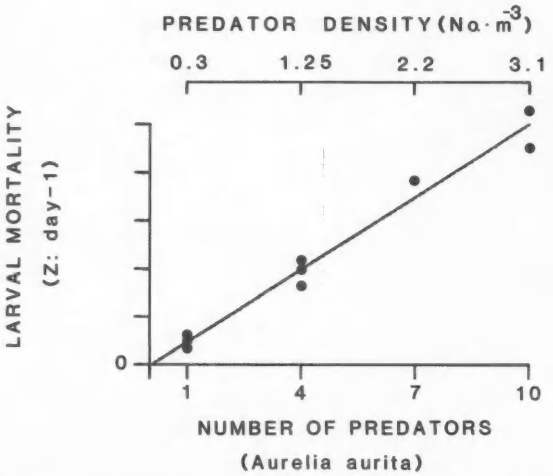

FIG. 4. Relationship between instantaneous larval mortality (Z) and the number of jellyfish (*Aurelia aurita*) in the enclosures. Regression equation is $Z = -0.0014 + 0.1266P$, $r^2 = 0.98$, $p = 0.0001$, $n = 4$.

during the first three experiments paralleled the increment in mean predator size (Table 3).

Larval mortality due to predation by the jellyfish *Cyanea capillata* was positively, but not significantly, correlated with the initial larval density. Mortality was, however, significantly ($p < 0.05$) correlated with predator size (Table 2). The slope of the log–log relationship between Z and predator diameter ($\ln Z = -2.578 + 1.067 \ln D$; $r^2 = 0.55$, $p = 0.05$) did not differ significantly from 1.0, indicating a linear relationship between these two variables. Standardized larval mortality rates (Zs) were not significantly correlated with initial larval density (Fig. 6A). The overall CV for Z and Zs was 37 and 26%, respectively.

Larval mortality caused by *Staurophora mertensi* was also independent of the initial density of larvae (Fig. 6B). Predator size was not significantly correlated with larval mortality (Table 2) and standardized mortality rates were also unrelated to larval density ($r^2 = 0.03$, $p = 0.63$).

In two series of experiments with replicated larval densities, larval mortality due to predation by *Staurophora mertensi* (Fig. 7A) and *Catablema vesicarium* (Fig. 8A) appeared negatively related to the initial larval density. The relationship was significant only for *Staurophora mertensi* (ANOVA, $F_{4,7} = 7.46$, $p = 0.01$) (*Catablema vesicarium*: $F_{5,10} = 1.47$, $p = 0.28$). However, because each level of replicated larval densities corresponded to experiments conducted at different dates, the initial density of larvae covaried with mean predator size in both species (Fig. 7 and 8). Consequently, the relationships between Z and initial larval density was probably subjected to the confounding effect of predator size. This possibility is supported by the fact that in both experiments, larval mortality rates were more strongly correlated with predator size than with initial larval density (Table 2).

The slope of the log–log relationship between larval mortality and mean predator diameter being not significantly (T-test, $p > 0.25$) different from 1.0 for either species indicated that the variables were linearly related. There was no relationship between initial larval density and standardized mortality rates for either predator (*Staurophora*: $F_{4,7} = 1.5$, $p = 0.25$, Fig. 7C; *Catablema*: ANOVA, $F_{5,10} = 0.96$, $p = 0.48$, Fig. 8C).

These two series of experiments provided estimates of the level of variation in mortality to be expected in experiments in which the initial larval density and other external variables are constant. In these experiments the CV's of replicated mortality rates ranged from 18 to 101% (mean 46%) for *Catablema vesicarium* and from 14 to 42% (mean 26%) for *Staurophora mertensi*. These values encompass those measured in our experiments with unreplicated larval densities (Table 3).

The lack of a significant relationship between larval mortality and initial larval density indicates that the predation rate of the four jellyfish species is linearly related to the number of larvae initially stocked (Table 4).

Effect of Alternative Food

No significant relationship was found between larval mortality rates (both Z and Zs) due to predation and initial larval density in five experiments in which alternative food was

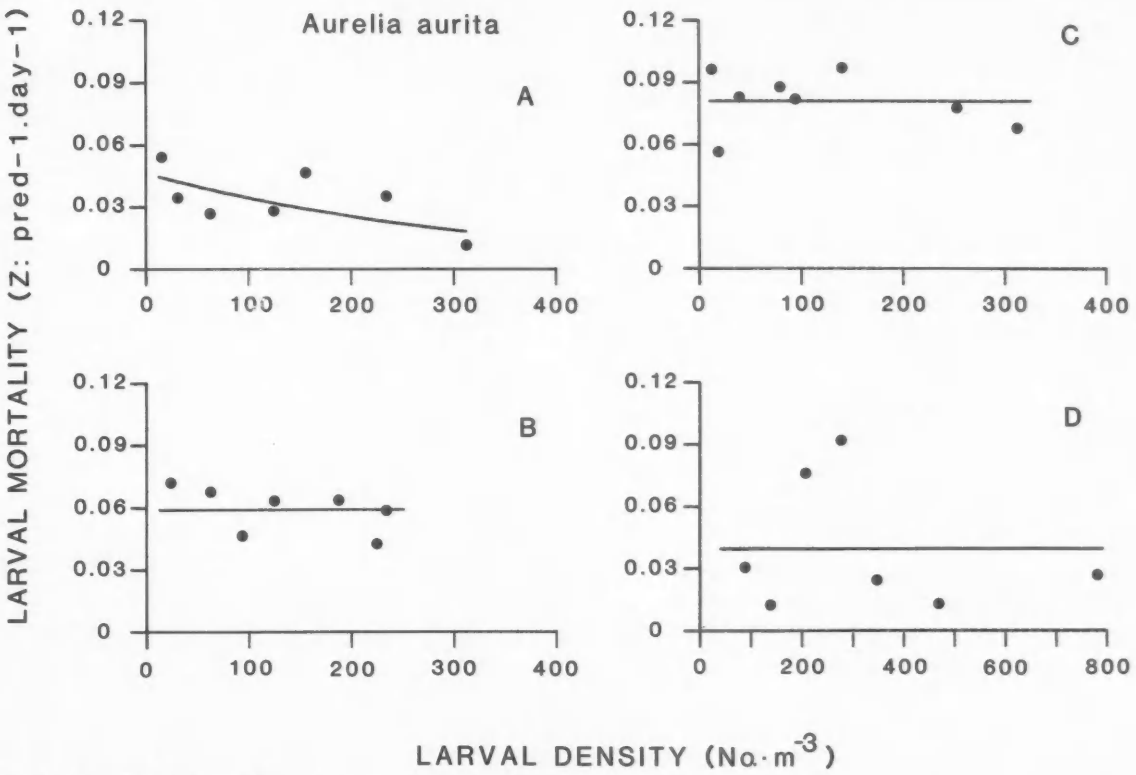

FIG. 5. Effect of initial larval density on larval mortality due to predation by the jellyfish *Aurelia aurita* in four different experiments. Letters indicate temporal sequence in experiments. Best fitted regressions are (A) $\ln Z = -3.073 - 0.0030L$, $r^2 = 0.44$, $p = 0.10$; (B) $Z = 0.068 - 0.000071L$, $r^2 = 0.29$, $p = 0.21$; (C) $Z = 0.084 - 0.000025L$, $r^2 = 0.04$, $p = 0.63$; and (D) $Z = 0.048 - 0.000027L$, $r^2 = 0.04$, $p = 0.65$.

TABLE 2. Product–moment correlation coefficients between larval mortality (Z), initial larval density (L), and predator umbrella diameter (D) for predation experiments. Logarithmic transformation ($\ln X$) was applied to some variables to evaluate potential curvilinearity. Significance levels: $*0.01 < p < 0.05$; $**0.001 < p < 0.01$.

Predator species	Figure	n	Z/L	$\ln Z/L$	$Z/\ln L$	$\ln Z/\ln L$	Z/D	$\ln Z/\ln D$	L/D
					Without alternative food				
A. aurita	5A	7	−0.60	−0.67	−0.60	−0.60	0.30	0.22	0.08
	5B	7	−0.53	−0.50	−0.59	−0.55	0.18	0.20	−0.01
	5C	8	−0.20	−0.16	−0.06	−0.01	0.54	0.58	0.49
	5D	7	−0.21	−0.15	−0.08	−0.07	−0.24	−0.06	−0.27
C. capillata	6A	7	0.68	0.64	0.61	0.60	0.76*	0.75*	0.88**
S. mertensi	6B	9	−0.16	−0.06	0.01	0.13	−0.59	−0.57	−0.15
S. mertensi	7A	12	−0.56	−0.69*	−0.68*	−0.80**	0.67*	0.78**	−0.76**
C. vesicarium	8A	16	−0.31	−0.19	−0.39	−0.23	0.42	0.42	−0.34
					With alternative food				
S. mertensi	9A	8	0.36	0.33	0.36	0.29	−0.03	−0.10	−0.19
	9B	5	0.27	0.28	0.40	0.41	−0.20	−0.17	−0.42
	9C	7	−0.07	0.03	−0.05	0.04	0.30	0.37	0.36
A. aurita	9D	7	0.42	0.44	0.46	0.46	0.68	0.71	0.66
	9E	7	0.09	0.18	−0.05	0.06	−0.50	−0.46	0.26

TABLE 3. Means and CV's (100·SD/mean) of instantaneous larval mortality (Z, predator^{-1}·d^{-1}) due to predation by four jellyfish species. Date, predator size (mean, CV, range of umbrella diameter), and water temperature (mean, CV, range) are listed for each experiment.

Experiment and predator species	Date	Larval mortality			Predator diameter (cm)				Water temperature (°C)			
		n	Mean	CV (%)	*n*	Mean	CV (%)	Range	*n*	Mean	CV (%)	Range
Predator density												
A. aurita	29 July 1984	9	0.122	14.9	42	6.85	19.9	4.00–10.57	41	9.3	9.8	8.0–11.3
Larval density												
C. vesicarium	1982[a]	16	0.055	63.2	80	1.70	21.7	0.70–2.52				2.8–8.8[a]
S. mertensi	1982[a]	12	0.140	66.6	51	6.13	46.4	2.38–12.32				6.0–10.5[a]
	4 Aug. 1983	9	0.131	38.7	36	12.99	15.3	8.55–17.16	40	10.1	14.8	7.1–11.9
C. capillata	21 July 1983	7	0.970	37.7	7	10.60	25.9	7.54–15.00	43	7.9	13.1	6.7–10.2
A. aurita	2 July 1983	7	0.034	40.9	21	2.60	19.4	1.91–3.60	41	8.2	15.3	5.8–9.8
	4 July 1983	7	0.059	18.4	28	3.17	19.2	2.17–4.80	40	7.4	18.1	5.8–9.6
	7 July 1983	8	0.081	16.9	32	3.75	15.8	2.20–4.68	43	8.7	10.5	7.3–10.4
	24 July 1983	7	0.039	80.4	14	7.06	17.9	5.38–8.76	38	8.5	24.2	5.2–10.6
Larval density with alternative prey												
A. aurita	17 July 1983	7	0.052	16.4	14	6.82	21.0	4.48–9.58	39	1.2	179.6	−0.9–4.8
	19 July 1983	7	0.147	41.2	14	6.32	26.9	3.76–10.74	39	5.5	48.8	0.4–9.3
S. mertensi	28 July 1983	8	0.053	60.1	32	9.38	21.5	5.33–14.27	37	7.0	22.4	3.8–9.3
	2 Aug. 1983	7	0.300	60.8	28	12.55	18.9	7.60–16.95	40	10.0	14.8	7.1–11.9
	6 Aug. 1983	7	0.127	28.8	28	14.11	15.1	8.67–18.06	39	8.5	12.0	5.9–9.9
Alternative prey density with fixed larval density												
A. aurita	13 July 1984	8	0.095	26.5	24	4.07	17.6	2.86–5.85	38	6.8	24.4	4.6–9.0
	25 July 1984	8	0.085	25.0	24	6.48	15.6	4.15–8.13	38	5.4	15.8	3.8–6.8
S. mertensi	23 July 1984	7	0.163	38.2	21	6.44	17.1	4.56–8.85	40	6.0	15.1	4.5–7.9
	27 July 1984	8	0.147	54.1	24	6.09	21.3	3.87–9.26	40	8.5	23.1	6.4–12.9
	31 July 1984	8	0.151	27.5	16	10.90	15.0	8.40–13.54	41	6.6	14.3	4.8–8.3

[a]Experiments conducted on different dates.

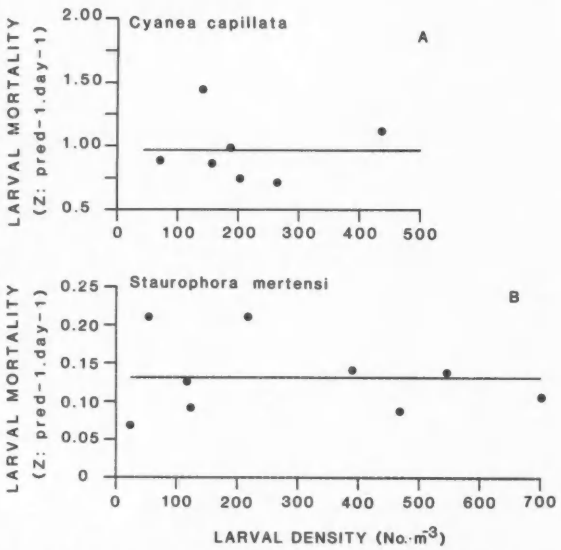

FIG. 6. Effect of initial larval density on larval mortality due to predation by (A) *Cyanea capillata* and (B) *Staurophora mertensi*. Regression equations are (A) $Z = 0.952 + 0.000069L$, $r^2 = 0.01$, $p = 0.95$; and (B) $Z = 0.140 - 0.000033L$, $r^2 = 0.03$, $p = 0.69$.

offered (Fig. 9). Two very low mortality rates observed at very high larval densities (>500·m^{-3}) during the second experiment with *Staurophora mertensi* may have been caused by the death of predators during the course of the experiment. These results clearly indicate that the addition of alternative food did not alter the linear form of the functional response for these jellyfish species (Table 4). The mean larval mortality rates varied significantly between experiments (time effect) for both *Staurophora* ($F_{2,17} = 141.3$, $p < 0.001$) and *Aurelia* ($F_{1,12} = 38.25$, $p < 0.001$). In addition to the predator size effect, temporal differences in the abundance and size distribution of the alternative prey added to the enclosures (Table 1) may also contribute to the between-experiment variability.

Experiments in which larval density was constant (80·m^{-3}) and alternative prey density was deliberately varied revealed no significant relationship between larval mortality and the initial abundance of alternative prey when employing *Staurophora mertensi* and *Aurelia aurita* as predators ($r^2 = 0.01$–0.18, $p = 0.29$–0.89, Fig. 10). Standardized larval mortality rates, calculated to account for the positive relationships between Z and predator size in two experiments, also showed no significant relationships with alternative food density ($r^2 = 0.01$–0.09, $p = 0.46$–0.97). Between-experiment differences in mean larval mortality were nonsignificant for both *Staurophora* ($F_{2,20} = 0.13$, $p = 0.88$) and *Aurelia* ($F_{1,14} = 0.65$, $p = 0.43$).

Effect of Predator Size

The relationship between Z and mean predator size (Table 2) was occasionally more significant than the controlled effect of either larval density (Table 2) or alternative food density. Moreover, the relationship between Z and mean predator size for both *Staurophora mertensi* and *Aurelia aurita* was variable, being

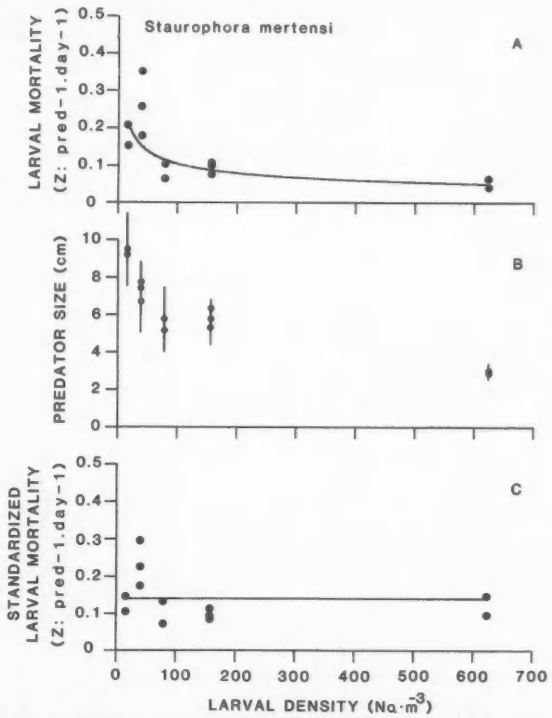

FIG. 7. Predation experiment with the jellyfish *Staurophora mertensi*. Larval mortality as a function of (A) initial larval density, (B) average predator size (umbrella diameter) at corresponding tested larval densities, and (C) standardized larval mortality as a function of initial larval density. Best fitted regression equations are (A) $Z = 0.369 - 0.0515 \ln L$, $r^2 = 0.46$, $p = 0.01$; and (C) $Zs = 0.152 - 0.000076L$, $r^2 = 0.07$, $p = 0.42$.

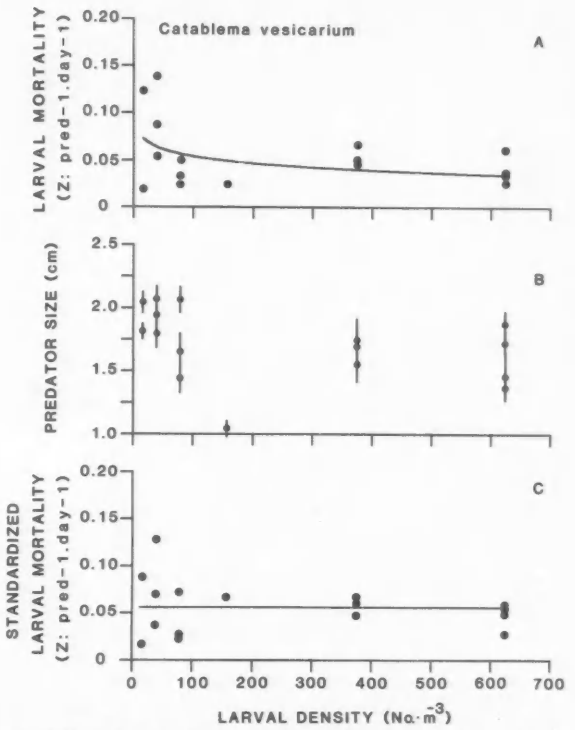

FIG. 8. Predation experiment with the jellyfish *Catablema vesicarium*. Larval mortality as a function of (A) initial larval density, (B) average predator size (umbrella diameter) at corresponding tested larval densities, and (C) standardized larval mortality as a function of initial larval density. Best fitted regression equations are (A) $Z = 0.102 - 0.0103 \ln L$, $r^2 = 0.32$, $p = 0.24$; and (C) $Zs = 0.059 - 0.000027L$, $r^2 = 0.13$, $p = 0.48$.

generally positive for small predators and negative for large predators.

Pooled results of experiments conducted in absence of alternative food revealed that the relationship between Z and predator diameter was nonlinear and dome-shaped for both predator species (Fig. 11A and 11B). A stepwise multiple regression analysis was performed to investigate, for both predator species, the effects of predator umbrella diameter, umbrella surface area, estimated wet weight, water temperature (both mean and standard deviation), and initial larval density on the variability in Z values. Initial larval size and condition were also added as independent variables in the regression for *Aurelia* only, larval length data being unavailable for 1982 experiments involving *Staurophora*. The data set for *Aurelia aurita* also included mortality rates obtained in the predator density experiment (Fig. 4) and results from the 3.2-m³ enclosures obtained in an experiment on the effect of container size (de Lafontaine and Leggett 1987b).

Regression models were highly significant ($p < 0.0001$) and explained 49 and 72% of the observed variability for *Staurophora mertensi* and *Aurelia aurita*, respectively (Table 5). For *Staurophora mertensi*, only mean predator size was found significant. For *Aurelia aurita*, variability in water temperature (standard deviation in temperature (SDT)) accounted for 50% of the overall variability in Z. Predator size explained 22% of the total variability (44% of the residual variance when the effect of temperature was removed).

Larval mortality estimates obtained from experiments in the

TABLE 4. Linear relationships between daily predation rate (I) of four jellyfish species and initial number of larvae (L) stocked into the enclosures in different predation experiments.

Predator species	Figure	$I = bL$ b	SE	r^2	F	p
A. aurita	5A	0.024	0.0053	0.74	20.48	0.004
	5B	0.053	0.0035	0.97	236.12	0.0001
	5C	0.073	0.0031	0.98	530.20	0.0001
	5D	0.029	0.0079	0.65	13.95	0.01
	9D	0.054	0.0021	0.99	635.63	0.0001
	9E	0.149	0.0121	0.95	152.30	0.0001
S. mertensi	7C	0.111	0.0038	0.99	829.16	0.0001
	6B	0.112	0.0083	0.95	181.73	0.0001
	9A	0.061	0.0128	0.73	22.73	0.002
	9B	0.342	0.0105	0.99	1061.11	0.0001
	9C	0.117	0.0045	0.99	671.36	0.0001
C. vesicarium	8C	0.044	0.0027	0.98	275.57	0.0001
C. capillata	6A	0.622	0.0309	0.98	403.70	0.0001

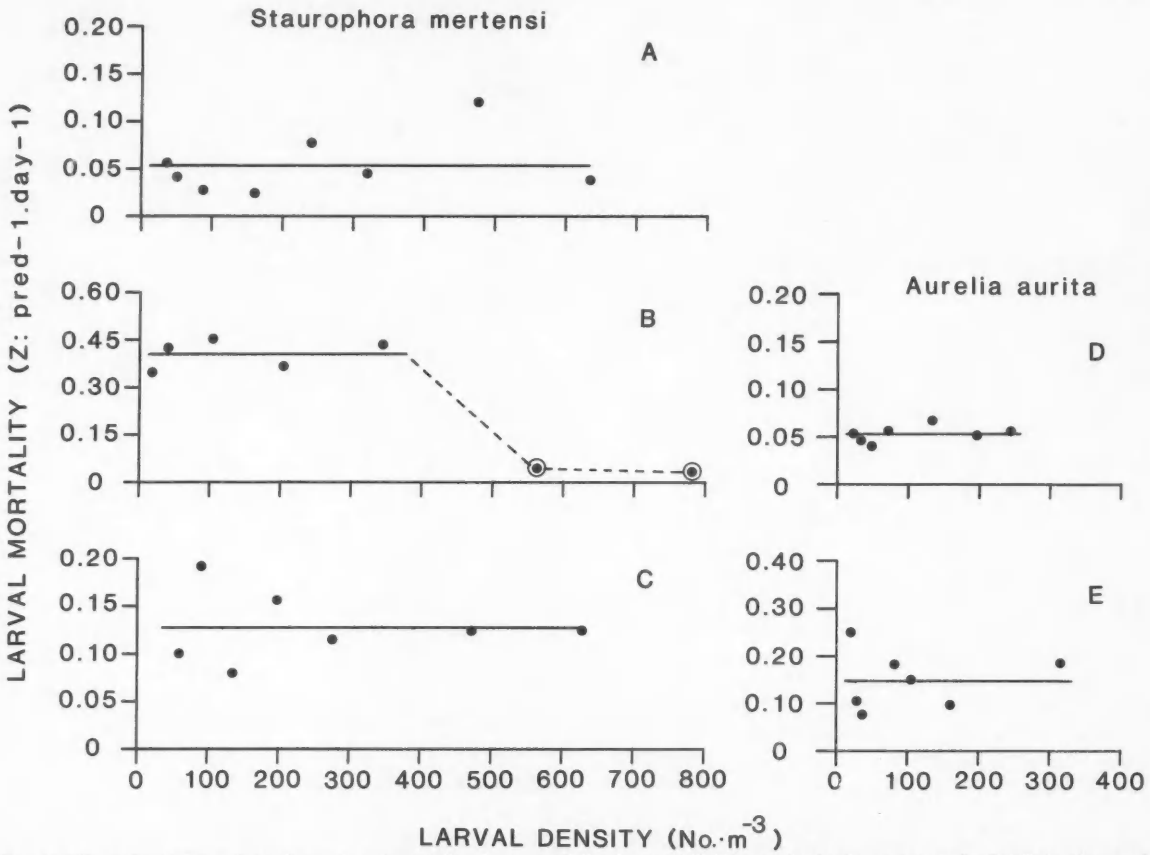

FIG. 9. Effect of initial larval density on larval mortality due to predation by two jellyfish species in the presence of a fixed abundance of alternative prey consisting of wild zooplankton. Letters indicate temporal sequence in experiments within each species.

presence of alternative food were added to the above data sets. The presence of alternative prey did not alter larval mortality due to predation by jellyfish (Fig. 11C and 11D). Despite the scatter induced by experimental conditions, the dome-shape relationship between Z and predator size remained evident. Stepwise regression analyses were repeated on the complete data set for each predator in including abundance (both in numbers and biomass) and mean size of alternative prey as independent variables. The absence of data on initial larval size for experiments conducted in 1982 resulted in the exclusion of 12 data points from the stepwise analysis for *Staurophora mertensi*.

Larval mortality was principally related to predator size and environmental conditions (Table 5). The models were significant ($p < 0.0001$) and explained 62 and 58% of the observed variability in larval mortality imposed by *Staurophora mertensi* and *Aurelia aurita*, respectively. In experiments involving *Staurophora mertensi* as predator, larval mortality was positively correlated with larval length which explained 30% of the overall variance. Variability in water temperature (SDT) during experiments, and mean temperature (which varied between experiments), contributed 14 and 8%, respectively. Predator size contributed 9.7% to the overall variance (20% of the variability when larval length and water temperature were stabilized). Despite the fact that the 1982 data were not included, the coefficient for the log-diameter variable was again very close to 2.0. This suggests that mortality rate was primarily a function of the umbrella surface area of *Staurophora mertensi*.

Larval mortality due to predation by *Aurelia aurita* was primarily related to variability in water temperature (SDT) and predator size which contributed 32 and 22% to the variability, respectively. The density of zooplankton added to the enclosures and its size composition explained only 4% of the overall variance in Z. Contrary to expectation, Z was positively related to the density of alternative prey ($p = 0.02$). The negative effect of average size composition was not significant ($p = 0.08$).

Product–moment correlations calculated to evaluate the potential covariability among variables showed the size of both *Staurophora mertensi* and *Aurelia aurita* to be significantly and positively correlated with the average size of alternative food (Table 6). Predator size was also positively correlated with temperature in the case of *Staurophora mertensi* and with larval size for *Aurelia aurita*. These positive correlations reflect the seasonal trend in these variables (Tables 1 and 3). A nonlinear regression technique (NLIN procedure, SAS Institute Inc. 1982) was employed to fit the following equation:

$$(4)\quad Z = a \cdot \text{size} \cdot \exp(-b \cdot \text{size}).$$

This second analysis differs from the stepwise analysis by forcing a specific relationship between larval mortality and predator size. Nonlinear models were significant for *Aurelia aurita* (r^2

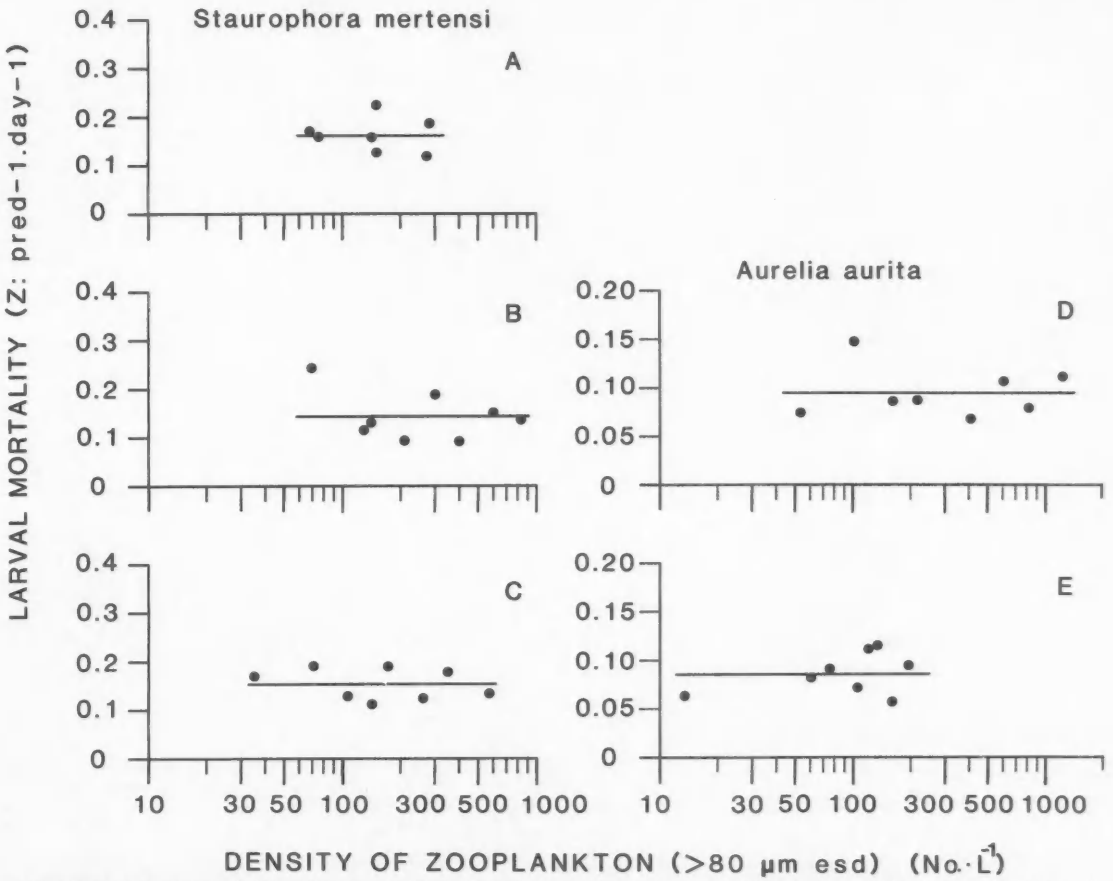

FIG. 10. Effect of abundance of alternative prey consisting of wild zooplankton on larval mortality due to predation by two jellyfish species at a fixed initial larval density. Letters indicate temporal sequence in experiments for each predator species.

= 0.25, p = 0.001), but not for *Staurophora mertensi* (r^2 = 0.09, p = 0.23). The residuals from the nonlinear models were examined to test the hypothesis that alternative food would reduce larval mortality. One-way ANOVA indicated no significant differences between mortality rates observed when alternative food was present or absent (*Staurophora*: p = 0.92; *Aurelia*: p = 0.27). A nonparametric test (Mann–Withney *U*-test) showed no relationship between mortality residuals and alternative food levels in experiments involving *Staurophora mertensi* (p = 0.72). For *Aurelia aurita*, a weak (p = 0.05) positive effect of zooplankton additions on *Z* was revealed. Residuals were, however, negatively related to variability in water temperature (SDT) in both cases and were positively related to larval size (Fig. 12) for *Staurophora*. These results were consistent with those derived from the exploratory stepwise regression analyses.

Discussion

This study represents the first intensive effort to experimentally evaluate larval fish mortality resulting from jellyfish predation at scales relevant to natural systems. CV's of larval mortality in replicated experiments varied between 11 and 101%, with the majority (72%) of the values being <50%. CV's in *Z* ranging from 11 to 50% correspond to CV's in predation rates (*I*) varying from 10 to 40%. Previously published laboratory studies have not explicitly reported the degree of variability in predation rates of different predators fed on larval fish. We therefore used published results to calculate the CV in predation rates measured in small laboratory containers. Replicated predation rates obtained in small (0.2–5 L) containers were highly variable and equivalent to those measured in our in situ enclosures (Table 7). We conclude that the small laboratory containers, whose use has been justified on the basis of the greater control that can be achieved under laboratory conditions, do not yield meaningfully better precision in estimates of predator feeding rates. This observation and the finding that container size has a strong influence on realized predation rates (de Lafontaine and Leggett 1987b) indicate that in situ enclosures of the type we employed provide equally reproducible and more realistic estimates of the larval mortality imposed by macrozooplankton predators.

Effect of Predator Density

The effect of jellyfish density on larval mortality has not previously been described. Larval fish mortality due to predation was linearly related to the density of jellyfish predators in enclosures (Fig. 4). This indicates that the feeding rate of

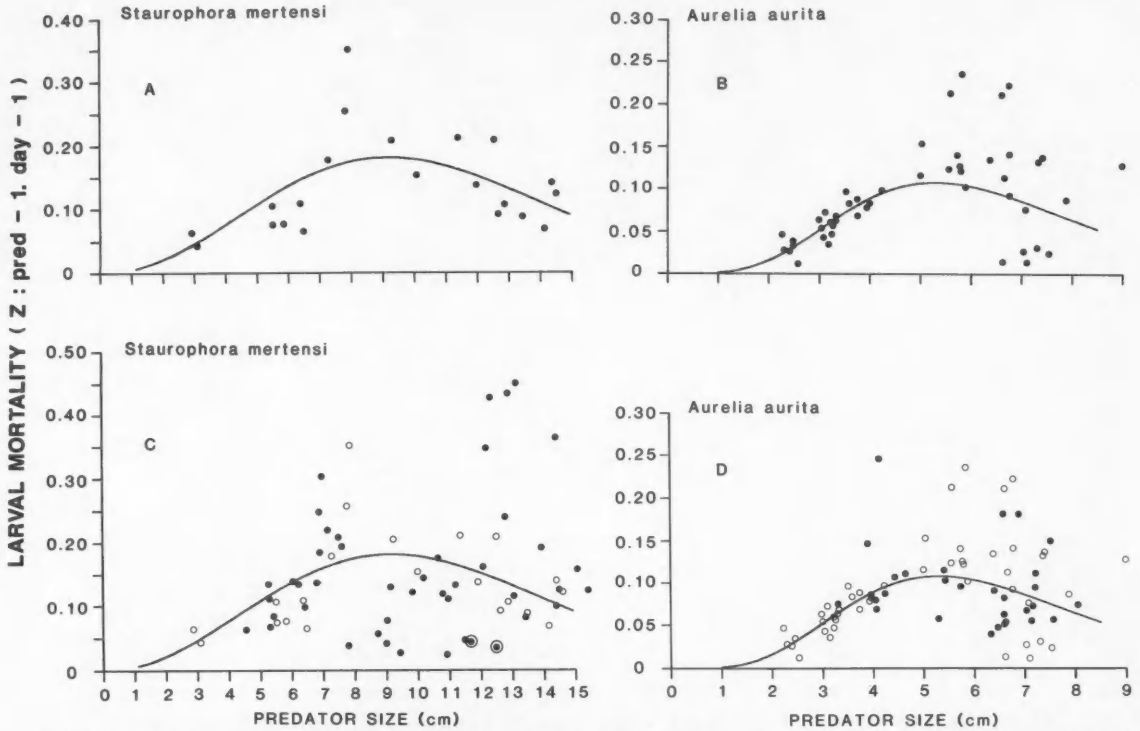

FIG. 11. Relationship between larval mortality (Z) and predator size for *Staurophora mertensi* and *Aurelia aurita*. Relationships are shown for (A and B) experiments without alternative food and (C and D) experiments in the presence (●) and absence (○) of alternative food. Curves depict the best fitted relationship (from Table 5) in the absence of alternative food (Fig. 11A and 11B) and are reproduced in Fig. 11C and 11D for visual reference of the effect of alternative food.

individual jellyfish was neither impaired (interference effect, Hassell et al. 1976) nor facilitated by the presence of conspecifics. Studies of copepods (Fabian 1960; Lillelund and Lasker 1971) and amphipods (von Westernhagen and Rosenthal 1976) feeding on fish larvae within small containers have shown that the larval mortality imposed by an individual predator decreased as the number of predators increased. von Westernhagen and Rosenthal (1976) argued that the decline in mortality rate per individual amphipod predator could be an artifact caused by more than one predator attacking the same prey. A similar observation was reported for copepods feeding in situ (Hartig et al. 1982). This situation is unlikely to occur with jellyfish predators that are larger than their larval prey and do not actively pursue their prey. Although different types of predators were investigated in these studies, the differences in the sizes of the experimental containers used might also explain the differences between their results and ours. In addition to the potential effect that confining large numbers of predators (up to 10 and 16) in small volumes (500 mL and 3.5 L) might have on their individual searching and feeding behavior, the use of small containers also caused rapid depletion of prey (85 and 100%) during some of these experiments (Fabian 1960; Lillelund and Lasker 1971). Mortality of this magnitude creates a serious problem in predation experiments in which ingested prey are not replaced (Murdoch and Oaten 1975; Arditi 1982; Houck and Strauss 1985) and may bias the effect of predator density. It is thus imperative, in these situations, to account for prey depletion in calculating the individual predation rate.

The densities of jellyfish predators used in our experiment varied between 0.3 and 3.0·m^{-3}. Such densities are not unusual for adult jellyfish which often appear in dense swarms (frequently >10·m^{-3}, Yasuda 1969; Möller 1980; van der Veer and Oorthuysen 1985). The relationship between larval mortality and jellyfish density revealed in our study indicates that these "blooms" can have a major impact on co-occurring larval fish populations. The selective occupation by fish larvae of water masses having lowered abundances of predators (Johannes 1978; Frank and Leggett 1982, 1983, 1985) is thus an effective strategy for significantly lowering mortality due to planktonic predators.

Effect of Larval Density

The finding that the magnitude of larval fish mortality imposed by jellyfish was independent of initial larval densities (Fig. 5–9) indicates that the feeding rates of jellyfish were never limited even when the capelin larval densities offered exceeded those reported in nature (>400·m^{-3}). We found no evidence of the Type II functional response that has been frequently reported for several other macrozooplankton predators (copepods: Lillelund and Lasker 1971; Bailey and Yen 1983; Turner et al. 1985; euphausiids: Theilacker and Lasker 1974; amphipods: von Westernhagen and Rosenthal 1976; von Westernhagen et al. 1979; Yamashita et al. 1984; chaetognaths: Kuhlmann 1977; ctenophores: Frank and Leggett 1982; jellyfish: Bailey and Batty 1983).

TABLE 5. Stepwise multiple regression models of larval mortality (Z) due to predation by *Staurophora mertensi* and Aurelia aurita. Results are presented for experiments in absence of alternative food only and for all experiments including those with alternative food. Best predictive relationships between Z and predator size (all other variables kept constant) are given.

Jellyfish	Variate	Coefficient (SE)	r^2	F	p
		Without alternative food			
S. mertensi	ln diameter	2.046 (0.514)	0.34	15.88	0.0009
(n = 21)	Umbrella area	−0.016 (0.005)	0.49	11.14	0.0037
	Intercept	−5.219			
	$\ln Z = -5.219 + 2.046 \ln \text{diameter} - 0.016 \cdot \text{area}$				
A. aurita	SDT	−1.206 (0.152)	0.50	68.10	0.0001
(n = 47)	ln diameter	2.815 (1.056)	0.69	7.10	0.0037
	Diameter	−0.435 (0.227)	0.72	5.12	0.0287
	Intercept	−3.143			
	$\ln Z = -4.789 + 2.815 \ln \text{diameter} - 0.435 \cdot \text{diameter}$				
		All experiments			
S. mertensi	Larval length	1.324 (0.319)	0.30	17.24	0.0001
(n = 52)	SDT	−1.116 (0.251)	0.44	19.74	0.0001
	Temperature	0.321 (0.081)	0.52	15.58	0.0003
	Area	−0.018 (0.005)	0.56	10.98	0.0018
	ln diameter	2.097 (0.790)	0.62	7.04	0.0109
	Intercept	−13.071			
	$\ln Z = -4.816 + 2.097 \ln \text{diameter} - 0.0185 \cdot \text{area}$				
A. aurita	SDT	−0.928 (0.145)	0.32	40.90	0.0001
(n = 77)	ln diameter	3.915 (1.047)	0.42	13.98	0.0004
	Diameter	−0.705 (0.221)	0.54	10.17	0.0023
	Total zoop.	0.0008 (0.0003)	0.56	5.65	0.0209
	Size zoop.	−0.063 (0.036)	0.58	3.07	0.0850
	Intercept	−4.001			
	$\ln Z = -5.034 + 3.915 \ln \text{diameter} - 0.705 \cdot \text{diameter}$				

TABLE 6. Product–moment correlation coefficients between measured variables in predation experiments. Note that the correlation matrix is a composite: the upper diagonal matrix is based on experiments with *Staurophora mertensi* (n = 64) and the lower diagonal matrix is based on experiments with *Aureliua aurita* (n = 77). Variables: larval density (Larvae), predator size (Diam.), water temperature (Temp.), variability in water temperature (SDT), larval length (Length), yolk-sac volume (Yolk), abundance of alternative food (Total), average size of alternative food (Size), and larval mortality (Z and ln Z). Significance levels: $*p < 0.05$, $**p < 0.001$.

	Larvae	Diam.	Temp.	SDT	Length	Yolk	Total	Size	Z	ln Z
Larvae	—	0.15	0.07	0.05	−0.33*	−0.02	−0.21	0.03	−0.22	−0.25
Diam.	0.13	—	0.43**	−0.12	−0.18	−0.56***	−0.25	0.53***	0.25	0.25
Temp.	0.19	−0.22	—	0.32**	0.01	−0.35*	−0.30*	−0.04	0.22	0.26
SDT	0.08	0.21	−0.54***	—	−0.19	0.47***	0.36**	−0.32**	−0.31*	−0.30*
Length	−0.13	0.36**	0.46***	−0.54***	—	−0.63***	0.27	0.24	0.32*	0.55***
Yolk	−0.16	−0.41***	−0.07	−0.07	−0.49***	—	0.22	−0.36**	−0.26	−0.48***
Total	−0.14	−0.09	−0.11	0.08	−0.14	−0.17	—	−0.05	−0.09	−0.04
Size	−0.19	0.32**	−0.63***	0.67***	−0.01	−0.33	0.06	—	0.34**	0.30*
Z	−0.13	0.25*	0.23*	−0.15	0.34**	−0.17	0.02	0.21	—	—
ln Z	−0.26*	0.22	0.14	−0.21	0.37**	−0.15	0.09	0.23*	—	—

The difference in predator functional response observed in our experiments relative to previous predation studies may be due to the size of containers employed. Most previous studies used small containers (<5 L) in which the number of larval fish offered corresponded to densities greatly in excess of that commonly observed in nature. Bailey and Batty (1983), who used densities equating to 7000–10 000 larvae·m^{-3}, have correctly noted that these high larval densities might occur in "patches" of fish larvae. However, no in situ densities approaching these levels have been reported so far. We have previously shown that small containers can cause larval mortality due to predation to be overestimated by at least one order of magnitude (de Lafontaine and Leggett 1987b). This large variability was attributed to differences in the relative distribution of both predators and prey in containers of different sizes. While it is theoretically possible that the feeding rate of these predators may reach saturation at very high densities of prey, our results suggest that at scales approaching those occurring in nature, it does

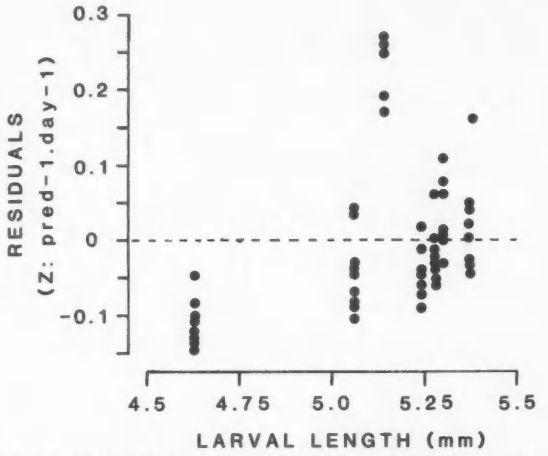

FIG. 12. Relationship between larval mortality (residuals from nonlinear regression) and larval length for experiments involving *Staurophora mertensi*.

not translate to such saturation effect at the level of a larval population. It appears that the probability of encounter between predators and prey is sufficiently reduced that, over time, a linear relationship between predation rate and the density of a larval population is to be expected under all but extremely high larval densities.

Prey depletion can also influence the functional response and lead to erroneous interpretations of the effect of prey density on predator feeding rate. Arditi (1982) mathematically demonstrated that the "averaging effect" of long-term experiments would result in a linearization of the functional response when predation rate is calculated as the number of prey taken per unit of time. This bias can be removed by calculating mortality rates from equations that account for prey depletion (Arditi 1982). Our calculation of mortality rate accounted for prey depletion, as recommended by Arditi (1982). The linear functional response observed in our experiments cannot, therefore, be attributed to an artifact caused by "averaging effect" of conducting 40-h experiments.

Frank and Leggett (1982), using enclosures similar to ours, found that mortality rates in larval capelin exposed to predation by ctenophores (*Pleurobrachia* sp.) were inversely related to the initial larval density. However, this relationship was derived by combining the results of several experiments conducted on different dates (K. Frank, Bedford Institute of Oceanography, Halifax, N.S., pers. comm). Our results clearly indicate that even relatively small between-experiment variations in predator size, larval size, and environmental conditions can seriously bias the interpretation of the observed relationship between larval mortality and larval density (Fig. 7 and 8). Since these potential sources of variability were not considered by Frank and Leggett (1982), the evidence for satiation in the case of ctenophore predators remains questionable. The feeding rates of the two ctenophore species (*Pleurobrachia pileus* and *P. bachei*) preying on copepods were linearly related to copepod abundance but the slope of the relationship varied as a function of predator size (Reeve et al. 1978; Greene et al. 1986).

The small container size typically used in previous predation experiments has required that small predators be used. These small predators may have limited capacity for ingesting larval fish because of limited gut capacity, slow digestion processes, long handling times, and/or low energy demands. Bailey and Batty (1983) reported that the predation rate imposed on herring larvae by jellyfish *Aurelia aurita*, averaging 1.3 cm in diameter, reached a maximum at larval densities ranging from 35 to 50 larvae·5 L^{-1} (equivalent to 7000–10 000 larvae·m^{-3}). These jellyfish were about 50% the size of the smallest *Aurelia aurita* used in our experiments. The possibility that small jellyfish become satiated at *naturally occurring prey densities* seems unlikely but remains to be verified.

The linear functional response exhibited by the four jellyfish species used in the present study is consistent with previous reports of the feeding rates of other gelatinous zooplankton (Reeve and Walter 1978; Reeve et al. 1978; Purcell 1982; Fulton and Wear 1985; Greene et al. 1986) preying on copepods. The linear (Type I) functional response is considered to be characteristic in filter-feeding organisms (Holling 1965; Murdoch and Oaten 1975; Poulet 1974, 1978; Conover 1978). Jellyfish do not actively pursue their prey and, while they are not strictly filter feeders, their feeding rate is primarily dependent on the probability of encounter between predators and prey.

Effect of Alternative Food

The presence of alternative prey did not alter the shape of the functional response exhibited by jellyfish (Fig. 9), and did not significantly reduce the instantaneous larval mortality rates imposed by *Staurophora mertensi* and *Aurelia aurita* (Fig. 10 and 11). This finding differs from theoretical expectations and from empirical observations of other predator–prey systems in which a shift in the functional response (generally from Type II to Type III) in the presence of alternative prey is common

TABLE 7. Range of CV's (100·SD/mean) in predation rates on larval fish measured in replicated experiments from various studies.

Predator type	Container volume (L)	CV (%)	Source(s)
Copepods	3.5	22–53	Lillelund and Lasker 1971
	1–4	23–78	Bailey and Yen 1983
	5	33–150	Bailey 1984
	1	7–43	Turner et al. 1985
Euphausiids	3.5	45–64	Theilacker and Lasker 1974
	5	20–200	Bailey 1984
Amphipods	0.5	1–18	von Westernhagen et al. 1979
	1	52–58	Yamashita et al. 1984
Jellyfish (<2 cm)	5	3–53	Bailey and Batty 1983; Bailey 1984
Jellyfish (>2 cm)	3200	10–63	This study

TABLE 8. Umbrella diameter, estimated wet weight, and average instantaneous larval mortality rate (Z, predator^{-1}·d^{-1}) due to predation by four jellyfish species. Mean, standard deviation (in parentheses), and range are given for each variable.

Jellyfish species	*n*	Umbrella diameter (cm)	Wet weight (g)	Mortality rate (Z)
Catablema vesicarium	16	1.70 (0.28) 1.04–2.07	2.17 (0.82) 0.55–3.39	0.055 (0.035) 0.018–0.137
Aurelia aurita	77	5.34 (1.74) 2.24–9.00	9.59 (7.16) 0.67–32.85	0.092 (0.052) 0.012–0.245
Staurophora mertensi	64	9.54 (3.38) 2.87–15.25	38.35 (30.95) 1.07–107.9	0.152 (0.099) 0.023–0.451
Cyanea capillata	7	10.60 (2.74) 7.54–15.00	127.8 (78.4) 40.5–301.5	0.969 (0.366) 0.632–1.587

(reviewed in Murdoch and Oaten 1975; Hassell 1978; Arditi 1982). There is, however, evidence that the linear functional response of filter-feeding copepods is unaltered by the presence of alternative food (Landry 1978; Roman 1984; Paffenhoffer 1984a, 1984b; Paffenhoffer and van Sant 1985). Our results for jellyfish are consistent with that finding.

The stability of jellyfish feeding rates on larval fish even when alternative food exceeded natural densities by 5 times (Fig. 10) contrasts with feeding studies involving copepods which have shown that ingestion rates of individual food items decrease in a mixture of prey (Poulet and Chanut 1975; Conover 1978; Landry 1981; Paffenhoffer 1984a, 1984b; Paffenhoffer and van Sant 1985). The hypothesis that higher abundance of zooplankton may be used as alternative prey and reduce larval mortality due to predation is not supported by our results. Pepin (1987) developed a simple foraging model which predicted that predation on larval fish is very sensitive to changes in abundance of zooplankton used as alternative prey by predators. This discrepancy may result from the linear functional response exhibited by jellyfish in our experiments and in previous studies (Reeve and Walter 1978; Reeve et al. 1978; Fulton and Wear 1985; Greene et al. 1986), while Pepin's model assumed a curvilinear (Type II) response. We conclude that Pepin's (1987) model should be applied to jellyfish predators only with extreme caution.

Factors Influencing Larval Mortality Rates

Our results indicate that neither prey density nor the presence of alternative food has a strong impact on larval mortality rates resulting from predation by jellyfish. Larval mortality was, however, strongly dependent on predator size, larval size, and environmental conditions during the experiments (Table 5). The effect of temperature variability on larval mortality due to predation is believed to be an artifact caused by conducting experiments at a fixed location subjected to short-term variability. The similar regression coefficients and the proportion of variance explained by SDT (Table 5) for experiments involving both *Staurophora mertensi* and *Aurelia aurita* suggest that the effect was similar in all experiments.

The dome-shape relationship between larval mortality and predator size observed for *Staurophora mertensi* and *Aurelia aurita* is atypical of other published studies in which a positive curve was generally observed (Theilacker and Lasker 1974; von Westernhagen et al. 1979; Daan 1986). Bailey and Batty (1983) and Bailey (1984) reported that the predation rates of *Aurelia aurita* were linearly related to the surface area of the umbrella.

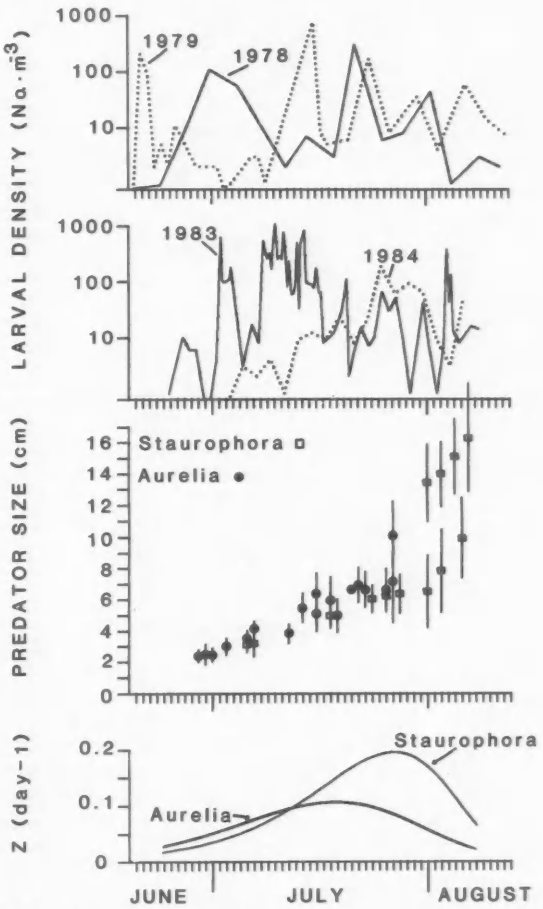

FIG. 13. Temporal variation of capelin larval abundance at Bryant's Cove, Nfld., during four years. Growth rate of *Staurophora mertensi* and *Aurelia aurita* (data pooled from 1982, 1983, and 1984) and estimated larval mortality rate due to predation. Larval abundance data for 1978 and 1979 are redrawn from Frank and Leggett (1981). Larval abundance data for 1983 were kindly provided by C. T. Taggart and those from 1984 are from Y. de Lafontaine (unpubl. data).

However, their conclusions were based upon the results of experiments in which a restricted size range (1.0–2.5 cm in diameter) of predators was employed. Our results are consistent with these findings when small jellyfish are considered. The decline in mortality rates as predator size increased has not previously been reported. The strong correlations between various measures of size (body diameter, surface area, and body weight) make it difficult to establish which of these variables is the principal determinant of the dome-shape relationship observed.

The 1-yr life cycle of *Staurophora mertensi* and *Aurelia aurita* is characterized by rapid growth during the medusan stage, culminating in reproduction and death near the end of the summer (Yasuda 1969; Möller 1980; Hernroth and Gröndahl 1983, 1985; van der Veer and Oorthuysen 1985). While the maximum size achieved prior to reproduction varies between locations and between years within an area (10–20 cm average), the release of gametes is known to induce pronounced physiological and morphological changes including tentacle reduc-

tion, diameter reduction, slower movement, and degeneration of feeding structures (Hamner and Jennsen 1974; Möller 1980; Fancett 1986). These factors may contribute to the reduced and more variable feeding rates we observed in large jellyfish.

The significant positive relationship between larval mortality and larval size observed in our experiments (Fig. 12) suggests that larger larvae, corresponding to late yolk-sac and early feeding larvae (Fig. 1), have an increased susceptibility to predation by jellyfish predators. It is unlikely that this increase in larval vulnerability with size resulted from active selection by jellyfish because size-selective mortality was not observed in individual experiments (Fig. 2). Increased larval vulnerability to predation during resorption of the yolk sac and the early feeding stages has also been reported for other predator types (von Westernhagen et al. 1979; Bailey and Yen 1983; Bailey 1984). However, our results do not permit differentiation if the increase resulted from higher predator encounter rate due to improved larval swimming ability or from reduced avoidance behavior due to poorer larval condition. Irrespective of cause, these findings indicate that larvae are more susceptible to predation mortality at the time of transition from endogenous to exogenous feeding. An increase in larval mortality at the time of yolk-sac resorption has frequently been sought as evidence for the starvation hypothesis (Marr 1956; May 1974; Dalhberg 1979). Such evidence has rarely been observed in situ (but see Fortier and Leggett 1985). Our findings indicate that even if repeated evidence of higher mortalities at this developmental stage is forthcoming, it would be premature to ascribe these losses solely to starvation effects. Our results also indicate that the hypothesis that larval mortality due to predation will be an inverse function of larval size (Cushing 1974; Ware 1975) is not general.

A comparison of the average larval mortality imposed by the four predator species (Table 8) reveals a positive relationship between larval mortality and the average body weight of the different predator species. The mean diameters of *Aurelia aurita*, *Staurophora mertensi*, and *Cyanea capillata* were very similar, but their average wet weights differed greatly. The greater mass of *Cyanea capillata* is mainly due to its larger tentacles and manubrium. The total biomass of alternative food added to the enclosures (mean = 1.52 g, range = 0.32–2.24 g) induced larval mortality rates (mean $Z = 0.062$, SE = 0.010) equal to those generated by experiments involving small *Catablema vesicarium* of equivalent total mass. While these data are insufficient to develop a general relationship, our results suggest that jellyfish feeding rates are more determined by individual size than by morphological differences in feeding appendages. This implies that the magnitude of predation losses to larval fish populations resulting from a complex of predators would be best estimated by a measure of the total biomass of potential predators rather than by an estimate of their numerical abundance.

Our finding that even very high larval densities caused no reduction in predation rates by jellyfish and that predation rate was directly proportional to predator numbers implies that reduced larval mortality due to jellyfish predation is thus achievable only by timing larval emergence to occur during periods when jellyfish are naturally reduced. This strengthens the hypothesis that the synchronous release of fish larvae in response to reliable environmental signals favoring the selection of specific water masses depauperate of predators may serve to avoid predation pressure (Johannes 1978; Frank and Leggett 1982, 1983, 1985). The adaptive strategy of synchronous release in response to predation pressure is interpreted as a result from selection of suitable environments. Because larval mortality due to predation is independent of larval density, the selection for high larval densities during a release event is not beneficial by itself. The "mass" occurrence is viewed as an obligatory result of the short-term releasing mechanism. It does not appear that the initiation of the larval drift is selectively adapted to minimize predation mortality via the functional response. The hypothesis that selection will favor to reduce species vulnerability to natural predators without the necessity of density-dependent mechanisms is conceptually similar to the "enemy free space" hypothesis recently proposed by Jeffries and Lawton (1984, 1985).

The pronounced effect of predator size on the resulting larval mortality observed in our experiments suggests that variations in the temporal occurrence of larvae relative to the seasonal cycle of predator size can lead to interannual variability in larval fish mortality and recruitment. An examination of the temporal pattern of abundance of capelin larvae in Bryant's Cove waters in the years 1978, 1979, 1983, and 1984 revealed that the timing of larval emergence was highly variable from year to year (Fig. 13). Larval releases induced by onshore wind events (Frank and Leggett 1981) are spread over a period of 30–40 d. Over the same time period, the jellyfish *Staurophora mertensi* and *Aurelia aurita* typically grow rapidly from an average size of 1–2 to 15–17 cm in diameter (Fig. 13). Using the relationship between larval mortality and predator size alone derived from our enclosures experiments (Table 5), we estimated the larval mortality rate imposed by these predator species as a function of time (Fig. 13). The temporal variability of mortality rates indicates that larval mortality due to jellyfish predation could vary between 5 and 20% per day due to variability in the emergence timing of capelin larvae relative to the growth trajectory of *Staurophora mertensi* and *Aurelia aurita*. Mortality due to predation is minimized when cohorts of larvae emerge while jellyfish are small (1979) and increases if the major releases are delayed in time (1984). It is clear that the chronology of interaction between larval emergence and predator growth can induce large variability in larval survival between years as well as within a year.

The mortality rates derived from our studies were calculated at an average predator density of $0.3 \cdot m^{-3}$. Such densities are high for a single species. Single species densities more typically range from 0.005 to $0.5 \cdot m^{-3}$ (Yasuda 1969, 1970; Möller 1980; van der Veer 1985; van der Veer and Oorthuysen 1985; Fancett 1986). However, the cumulative impact of two or more coexisting jellyfish species could easily induce larval mortalities equivalent to those we calculated. Taggart and Leggett (1987) and Fortier and Leggett (1985) estimated that daily mortality of larval capelin averaged 40–60% during the first days following their release. Our results, and the observation by Taggart and Leggett (1987) that predator density was positively correlated with intra- and interannual variability in larval mortality, suggest that macroinvertebrate predation has the potential of being the primary regulator of larval survival, as has previously been proposed.

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Feeding of Juvenile Coho Salmon (*Oncorhynchus kisutch*): Maximum Appetite, Sustained Feeding Rate, Appetite Return, and Body Size

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Experimental investigations of the influence of body size and feeding rate on the voluntary consumption of live *Tubifex* by juvenile coho salmon (*Oncorhynchus kisutch*) showed that maximum appetite and sustained feeding rate increased allometrically with coho body size. There was no significant effect of body size on the rate of appetite return following a meal, although a comparison of these results with data from the literature suggests that the rate constant of appetite return may decrease with body size. The rate constant of appetite return following meals preceded by 96 h of starvation was significantly greater than that for meals preceded by 3–5 d of satiation feeding. If the latter effect also applies to the rate constant of gastric evacuation for live foods, the use of standard gastric evacuation models could overestimate the rate of food intake of wild fish feeding at close to their maximum rate.

Les résultats d'expériences sur l'influence de la taille corporelle et du taux d'alimentation du saumon coho (*Oncorhynchus kisutch*) juvénile sur la consommation volontaire de *Tubifex* vivant ont révélé que l'appétit maximum et le taux d'alimentation soutenu augmentent de façon allométrique en fonction de la taille corporelle du coho. On n'a pas observé d'incidence significative de la taille corporelle sur le taux de retour de l'appétit après un repas, quoiqu'une comparaison des résultats obtenus avec des données publiées porte à croire que la constante du taux de retour de l'appétit peut diminuer en fonction de la taille corporelle. La constante du taux de retour de l'appétit après la prise de nourriture précédée d'une période de privation de 96 h était nettement plus élevée que celle observée après des repas précédés de 3 à 5 d d'alimentation à satiété. Si cette dernière observation s'applique aussi à la constante du taux d'évacuation gastrique de proies vivantes, des modèles normalisés d'évacuation gastrique pourraient surestimer le taux de prise de nourriture chez les poissons sauvages qui s'alimentent à un taux proche de leur taux minimum.

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The maximum amount of food that fish can consume, either in a single meal (maximum appetite) or at a sustained rate, has both practical and ecological importance. Fish grown under culture are typically exposed to high food availability, and, for a particular meal frequency, their feeding rate will be determined by maximum appetite and the rate at which appetite returns following a meal. Under such conditions, information on maximum appetite, the rate of appetite return, and how these vary with body size and feeding frequency can be useful in the optimization of feeding schedules. Wild fish generally encounter lower food densities, but for forms such as juvenile salmonids, which exploit periodically abundant invertebrate drift or plankton, appetite may at times limit food intake, potentially influencing ecological correlates of feeding rate such as intra- and interspecific competition or predator–prey interactions.

Maximum appetite is closely related to stomach volume which, as a morphological feature, would be expected to vary more or less isometrically with body size. When stomach volume is measured by injecting water under pressure into ligatured stomaches, this appears to be the case (Jobling et al. 1977; Flowerdew and Grove 1979; Jobling 1980). Surprisingly, however, maximum appetite, which is based on voluntary food intake, shows an allometric scaling similar to that of standard metabolism (Brett 1971; Elliott 1975a). In salmonids, maximum sustained feeding rate also scales allometrically with body size (Brett 1971; Elliott 1975b), possibly because of its relationship to maximum appetite. However, the scaling of maximum feeding rate with body size under a particular feeding regime may also depend on the body size scaling of the rate constant of appetite return following a meal.

There is an inverse relationship between voluntary meal size (appetite) and stomach fullness prior to feeding (e.g. Brett 1971), implying a corresponding relationship between the rate constants[2] of appetite return following a meal and gastric evacuation.

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[2]The rate of a process, such as gastric evacuation, is distinguished from its rate constant as follows. Let stomach fullness at time t equal X; then dX/dt is the *rate* of gastric evacuation, while if $dX/dt = -Rf(X)$, where R is a constant, then R is the *rate constant* of gastric evacuation.

Calder (1984) has argued that the time required for animals to process their food should scale with body size as does physiological time (i.e. as (body mass)$^{0.25}$). By extension, the rate constants for processes determining food turnover time, including gastric evacuation (and appetite return), would scale as the inverse of physiological time (i.e. (body mass)$^{-0.25}$). Studies indicate that the rate constant of gastric evacuation does decline with increasing size, but only at the small end of the size spectrum (e.g. Elliott 1972; Flowerdew and Grove 1979; Brodeur 1984; Mills et al. 1984; Talbot et al. 1984). It is not known if the rate constant of appetite return follows a similar pattern.

The influence of the rate of appetite return on feeding rate could be complicated by a potential interaction with meal frequency. Noble (1973) suggested that the rate constant of gastric evacuation could be reduced at high feeding rates because of inhibitory effects of partially digested food in the gut. A reduction in the rate constant of gastric evacuation would lead to a similar reduction in the rate constant of appetite return. The information bearing on changes in either of these rate constants with meal frequency in salmonids is equivocal. Using pelleted food, Talbot et al. (1984) found no change in the rate constants of gastric evacuation after repeated feeding in Atlantic salmon (*Salmo salar*), while Brett's (1971) data showed no effect of repeated feeding on the rate constant of appetite return in young sockeye salmon (*Oncorhynchus nerka*) fed pellets twice daily for a period of 12 d. In experiments in which brown trout (*Salmo trutta*) were fed fresh *Gammarus*, Elliott (1975b) found no difference in the size of four meals consumed over 24 h. In contrast, Godin (1981) reported that pink salmon (*O. gorbuscha*) continuously supplied with fresh copepods ad lib for 12 h following a satiation meal consumed these at a lower rate if fed prior to the satiation meal, results consistent with the effect proposed by Noble (1973). Although inhibition of appetite gain or gastric evacuation would seem more likely with live foods due to their initially higher rate of gastric evacuation (e.g. Brett and Higgs 1970; Elliott 1972), no experimental work with live food has been carried out over a time course comparable with that used in Brett's (1971) work on pelleted diets.

The present study extends the experimental analysis of feeding processes in salmonids through an examination of two aspects of appetite allometry and dynamics in juvenile coho salmon (*O. kisutch*): (1) effects of body size on maximum appetite, sustained feeding rate, and the rate constant of appetite return and (2) the response of appetite return to repeated satiation feeding, but over a longer time course than in previous studies using natural foods.

Materials and Methods

The fish used in these experiments were obtained by seine from Widgeon Creek, a tributary of the lower Pitt River, British Columbia, in four collections made in October and November 1977, May 1978, and April 1979. Fish lengths ranged from 40 to 80 mm depending on collection date. Water temperature in Widgeon Creek at the time of capture varied from 6 to 15°C. In the laboratory, fish were held individually in right or left halves of 45-L aquaria bisected by opaque partitions. A continuous flow of dechlorinated water (12°C) was supplied to all tanks, and lighting (12 h light : 12 h dark) was provided by overhead fluorescent tubes. Fish resumed feeding within 1–2 d following capture. All fish received a daily ration of live worms (*Tubifex* sp.; approximately 3–5% of fish wet weight) during the preexperimental period which lasted for a minimum of 30 d. This period was chosen arbitrarily but appeared sufficient to acclimatize fish to the experimental holding facilities. Prior to the initiation of feeding experiments, fish were starved for 96-h to ensure that previously ingested food had been cleared from their digestive tracts.

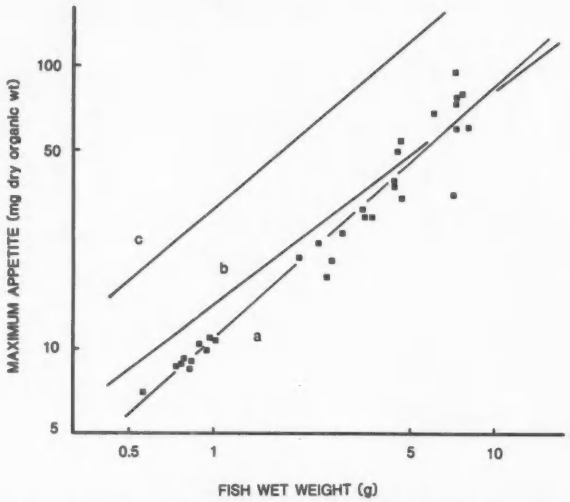

FIG. 1. Appetite after 96 h of starvation as a function of fish wet weight for 31 fish ranging in weight from 0.5 to 8.2 g. Line *a* is the least squares regression line for the log-transformed data ($\log_{10}$ (meal size) = 1.03 + 0.88 $\log_{10}$ (fish weight)). Line *b* is Elliott's (1975a) for brown trout ($\log_{10}$ (meal size) = 1.154 + 0.76 $\log_{10}$ (fish weight)). Line *c* was calculated from Brett's (1971) data for sockeye salmon feeding on pelleted food at 15°C (meal size = (fish weight) {29.9 − [10.6 $\log_{10}$ (fish weight)]}). The equation for line *c* is of a different form because Brett (1971) expressed meal size as % body weight.

For experimental meals, a measured quantity of *Tubifex* (mixed sizes), in excess of the amount finally consumed, was introduced to each tank half and the fish allowed to feed for 15 min. Although preliminary trials indicated that this length of time was sufficient for most fish to reach satiation, worms introduced at the water surface subsequent to this period sometimes elicited feeding responses as they sank even when those on the tank bottom did not. Therefore, following the initial 15-min feeding period, additional worms were introduced at approximately 30-s intervals. When no feeding response was observed as these worms sank to the bottom, fish were considered satiated. All remaining worms were then removed by siphon, weighed, and this value subtracted from the total weight of worms introduced to give the meal size. To facilitate comparison with other studies, meal wet weights were converted to dry organic weights using the conversion factor (0.12) obtained by Elliott (1972) for terrestrial oligochaetes. Experimental feeding was continued for a total of 5 d (three meals daily, provided as described above, at approximately 0900, 1330, and 1800). At the end of this period the fish were starved for 96 h and weighed. A total of 31 fish (0.5–8.2 g; 40–80 mm) were used in this experiment.

Results

Body Size Effects

Maximum appetite

Maximum appetite was defined as the size of the initial meal (meal 1, day 1, 96 h of starvation). Regression of $\log_{10}$ initial

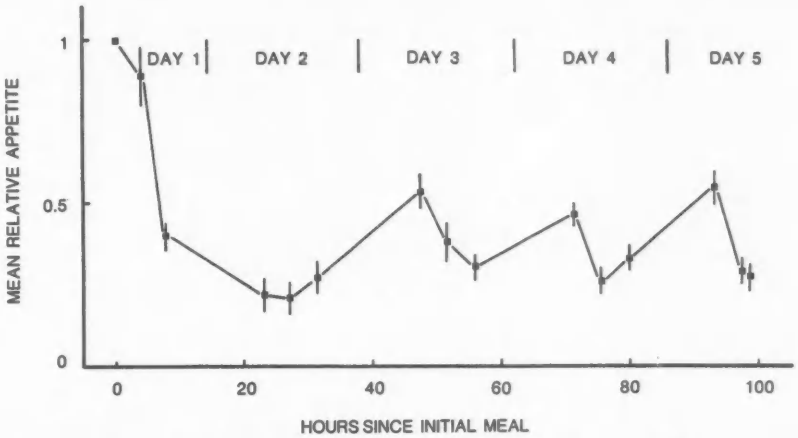

FIG. 2. Appetite (relative to the size of the initial meal) as a function of time from the first meal. The data are pooled for all fish (n = 31). Vertical bars represent 1 SE. Since the timing of the meals was not exactly the same for all fish, the time of a meal is actually a mean value.

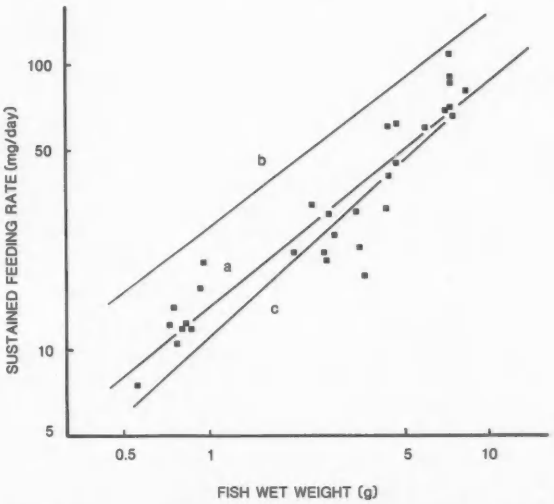

FIG. 3. Maximum sustained feeding rate (dry organic weight) as a function of fish wet weight for 31 fish ranging in size from 0.5 to 8.2 g. Line *a* is the least squares regression line for the data ($\log_{10}$ (feeding rate) = 1.14 + 0.79 $\log_{10}$ (fish weight)). Line *b* is Elliott's (1975b) for brown trout ($\log_{10}$ (feeding rate) = 1.42 + 0.76 $\log_{10}$ (fish weight)). Line *c* is maximum appetite (= line *a* in Fig. 1).

meal size against $\log_{10}$ fish wet weight (Fig. 1) shows that maximum appetite increased with body weight, but at a decreasing rate (slope = 0.88).

Sustained feeding rate

Sustained feeding rate, i.e. the maximum feeding rate that can be sustained indefinitely under a particular feeding schedule, is calculated as the ratio of total food consumed over a time interval to the interval length. However, because this metric could be sensitive to changes in feeding behaviour during the experiment, the temporal variation in the results was first examined for evidence of such changes. To do this, the meal sizes for each fish were divided by that fish's initial meal size. The resulting standardized data sets (one for each fish) were then treated as replicates and a mean meal size calculated for each meal. The temporal pattern of these standardized means (Fig. 2) reveals the expected positive relationship between appetite (meal size) and food deprivation time. Not only was the initial meal the largest meal consumed during the experiment, but, from day 3 onward, the first daily meal (following 14.2–16 h of starvation) was larger than the two subsequent meals (2.7–5 h of starvation) which were similar to each other.

The problem of estimating sustained feeding rates is complicated by the sharp decline in the daily ration after day 1, when the two largest meals were consumed (Fig. 2, day 1, meals 1 and 2). In particular, the appetite gain after meal 1 (day 1), indicated by the size of meal 2 (day 1), was much higher than for any subsequent intermeal interval of similar duration. If, as this suggests, the rate of appetite return following a meal depends on the timing of a number of previous meals, it would be incorrect to include, in the calculation of sustained feeding rate, food ingested before the rate of appetite return had stabilized. Because of the apparent decline in the rate constant of appetite return over the first 2 d of the experiment, the sustained feeding rate for each fish was obtained by dividing its total food consumption in all experimental meals following meal 1 on day 3 by the total time between this meal and the last meal of the experiment (meal 3, day 5). Regression of this value against fish weight ($\log_{10}$-transformed data) shows that sustained feeding rate increased with fish size at a decreasing rate (Fig. 3, line *a*), although with a flatter slope than was the case for maximum appetite (Fig. 3, line *c*).

Rate of appetite return

To investigate the relationship between body size and the rate of appetite return following a meal, a method for describing this process was required. Although a lag in appetite return may occur immediately following a satiation meal without an accompanying lag in the evacuation of stomach contents (Brett 1971; Elliott 1975b; Grove et al. 1985), the size of a meal and stomach fullness just prior to that meal have been shown to be closely related (Brett 1971; Elliott 1975b; Godin 1981; Grove et al. 1985). Preliminary experiments with coho revealed an initial lag in appetite following satiation feeding (after 96 h of starvation), but this effect disappeared within 2 h postfeeding.

TABLE 1. Comparison of the hourly rate of appetite return (G) for meal 1, day 1 and meals 1 and 2, days 3–5.

Meal category	Mean intermeal interval in hours (sample size)	Mean G (SE)
Meal 1, day 1	3.9 (30)	0.48 (0.04)
Meals 1 and 2, days 3–5	4.2 (137)	0.11 (0.008)

Therefore, for intermeal intervals greater than 2 h, the time course of appetite return following a meal should reflect that of stomach fullness, which, for relatively small foods such as *Tubifex*, is considered to decrease exponentially as gastric evacuation proceeds (Jobling 1986; Persson 1986).

The potential appetite, A_t, at time t following a satiation meal will vary from 0 at $t = 0$ to A_m, where A_m is the maximum appetite. The difference $A_m - A_t$, the amount by which appetite could still increase if feeding did not take place at time t, is analogous to stomach fullness at time t. By further analogy to gastric evacuation, the change in $A_m - A_t$ with time can be treated as an exponential process with rate G to yield the following relationship:

$$d(A_m - A_t)/dt = -G(A_m - A_t)$$

or since $dA_m/dt = 0$:

$$(1) \quad dA_t/dt = G(A_m - A_t).$$

Solving (1) for G for meal i (G_i) gives

$$(2) \quad G_i = (\log A_m - \log (A_m - A_t))/t$$

and for any meal i, G_i can be estimated from the maximum appetite (A_m) and the size of meal $i + 1$ at time t following meal i (A_t). In applying the above model to the appetite data, A_m's for each fish were equated to the largest meal consumed by that fish and equation (2) was used to calculate G_i's for two meal categories with comparable t values: (i) meal 1, day 1 and (ii) meals 1 and 2, days 3–5. G_i's were not calculated if the size of meal i or $i + 1$ was zero. An analysis of variance performed on the log-transformed data showed no significant effect of fish size on G for meal categories (i) ($F_{1,28} = 1.15$) or (ii) ($F_{30,108} = 1.05$).

Influence of Repeated Satiation Feeding on the Rate of Appetite Return

The results of the previous section suggest that G, the rate constant of appetite return, is independent of fish size. Therefore, to investigate the effect of repeated satiation feeding on G, the G values for all fish were pooled separately for meal categories (i) and (ii) above to produce two means (Table 1). The mean G for category (i) was significantly greater than that for category (ii) (t-test; $p < 0.001$; df = 167).

Discussion

The increase in maximum appetite with body size for juvenile coho salmon (Fig. 1) is comparable with trends reported for other salmonids. Elliott (1975a) found a similar relationship for brown trout feeding on a number of organisms including oligochaetes (Fig. 1, line *b*). Brett's (1971) results for sockeye salmon (Fig. 1, line *c*) parallel the coho data with respect to slope, although maximum appetite for sockeye is larger by a factor of 3. While this may in part reflect a difference in the stomach volume of sockeye and coho, it is more likely a consequence of sockeye being able to consume considerably larger meals, in terms of dry organic weight, when eating the dry food pellets used in Brett's (1971) study.

Sustained feeding rate was also found to increase allometrically with body size (Fig. 3). The only comparable salmonid data are Elliott's (1975b) for brown trout feeding on *Gammarus*, which show a similar slope for this relationship (Fig. 3, line *b*) but, because of a higher intercept, predict that brown trout would feed at almost twice the rate of similar sized coho. A possible explanation for this is discussed below.

Although there are theoretical reasons to believe that the rate constant of appetite return might scale with body size as the inverse of physiological time (i.e. as (body mass)$^{-0.25}$; see the introduction), in this study the relationship between $\log_{10}$ body size and $\log_{10} G$ was not significant. However, the expected slope of this relationship (-0.25) is relatively flat and this result may have been due to the high variance in the appetite data and the limited range of body sizes used in the experiments rather than to the absence of a body size effect on the rate constant of appetite return. To extend the body size range, the G values obtained here could be compared with gastric evacuation rates from similar studies using larger fish. When this was done for Elliott's (1972) brown trout data, it was found that the pooled value of G (meal 1, day 1) for coho with a mean body size of 2.76 g (0.48/h; Table 1) is substantially greater than the rate constant of gastric evacuation for 90-g brown trout (0.21/h) under similar feeding and temperature conditions. Despite the fact that appetite may not be an exact correlate of stomach fullness (see above), the slope of the line joining the log transforms of the points (2.76 g, 0.48) and (90 g, 0.21) is -0.24, close to the weight exponent for physiological rate constants suggested by allometry. The contrasting rate constants of appetite return for 3-g coho and gastric evacuation for 90-g brown trout are consistent with declines in rate constants of gastric evacuation with body size reported for small size classes of other species (e.g. Mills et al. 1984; Flowerdew and Grove 1979). In larger fish, a relationship between the rate constant of gastric evacuation (or appetite return) and body size has not been demonstrated (Elliott 1972; Brodeur 1984; Talbot et al. 1984).

Additional information on the relationship between body size and G can be obtained indirectly through comparisons of the weight exponents of the regressions of maximum appetite and sustained feeding rate on body weight. If fish fed continuously so as to maintain maximum stomach fullness, their sustained feeding rate would equal GA_m, where A_m is the maximum appetite (Fig. 1, line *a*). Alternatively, if feeding frequency, F, is sufficiently low that fish evacuate most of their previous meal prior to their next meal, sustained feeding rate would approach FA_m. If G is independent of size, sustained feeding rate would scale with weight as does A_m, i.e. as W^{α}, where α is a positive constant (see Fig. 1), regardless of meal frequency. However, if G declines with body size as suggested above, say as W^{-x}, the weight scaling of sustained feeding rate would increase from $W^{\alpha - x}$ for continuous feeding to W^{α} for low-frequency feeding. An intermediate scaling, somewhat less than W^{α}, would be expected for the feeding frequencies used in this study. There is some indication of this, as the weight exponent for sustained feeding rate is less than that for maximum appetite (0.79 vs. 0.88), as expected if G declines with body size and feeding is relatively frequent; however, this difference is not significant (t-test; $p < 0.2$; df = 58). Data from other studies are similarly ambiguous on this point. Brett's (1971) results for young sock-

eye salmon also indicate that the body weight exponent for sustained feeding rate is smaller than that for maximum appetite (~0.64 vs. ~0.84), while Elliott (1975a, 1975b) found that these had identical weight exponents (0.76) in brown trout.

There was a significant difference between the mean rate constant of appetite gain, G, following meal 1, day 1 and that following meals 2 and 3 on days 3–5. Appetite gain and gastric evacuation are linked; therefore, the decline in G following day 1 could reflect a similar decline in the rate constant of gastric evacuation. Noble (1973) observed that perch evacuated food ingested at the beginning of a meal more rapidly than that which they ingested following the meal. Based on this, Noble (1973) suggested that the filling of the hindgut with partially digested food following repeated satiation feeding could inhibit the evacuation from the stomach of subsequent meals. However, Noble's result could simply be due to foods being evacuated in a serial fashion (i.e. in the order in which they were consumed) rather than to inhibition of gastric evacuation. Because any reduction in the rate constant of gastric evacuation would be reflected in the rate constant of appetite return, better evidence for the effect suggested by Noble comes from the results of the present study. Such an inhibitory effect could serve to maintain food in the gut for a length of time sufficient to ensure that food assimilation efficiency remain high even at high feeding rates.

If the rate constant of gastric evacuation, say R, can vary with the feeding rate, this could have implications for the study of fish feeding energetics. For example, rates of gastric evacuation are usually derived from experimental data documenting the time course of stomach fullness following a satiation meal which was preceded by a long period of starvation (e.g. Brett and Higgs 1970; Tyler 1970; Jobling and Davies 1979). However, if the rate constant of gastric evacuation is a decreasing function of feeding rate, the use of a nonvarying parameter for calculating daily food intake from stomach fullness data (e.g. Elliott and Persson 1978; Pennington 1985) could lead to overestimates if fish are feeding at rates close to their maximum. Although high feeding rates are more typical of fish grown under culture, the extension of the results obtained using live *Tubifex* to feeding practices in fish husbandry is problematic, since dry commercial feeds would have different gastric evacuation characteristics.

Dill (1983) has shown that when R is independent of sustained feeding rate, there will be a linear relationship between feeding rate and equilibrium stomach fullness. However, if, as suggested above, R declines as sustained feeding rate increases, this relationship would become an accelerating one, i.e. equilibrium stomach fullness would increase out of proportion to sustained feeding rate. This could have ecological consequences if there is an energy cost associated with transporting food held in the stomach or if a fish's visibility, and hence risk of predation, increased with its stomach fullness or distention. This would be particularly important for transparent pelagic larval fishes, as they are largely invisible except for the contents of their stomachs. An increase in predation risk with gut fullness has been demonstrated for the transparent pelagic larvae of the phantom midge *Chaoborous* (Giguère and Northcote 1987). Recent work done by Abbott et al. (1985) identifies a somewhat different fitness cost that some juvenile salmonids may incur as a consequence of having a full stomach. In their study, dominant steelhead trout (*Salmo gairdneri*) were more aggressive towards a subordinate when the subordinate's abdomen was distended with food. Costs, such as those outlined above, would tend to discount the per unit value of food at higher feeding rates, relative to the case of constant R, possibly favouring a feeding rate, and equilibrium stomach fullness, less than the potential maximum when food is continuously abundant. However, fish would still be expected to utilize the full storage capacity of their stomaches when food is only abundant periodically, since any decrease in R would only occur at high *sustained* feeding rates.

A decline in the rate of gastric evacuation accompanying repeated satiation feeding could account for the twofold difference in the sustained feeding rates predicted for coho and brown trout (Fig. 3, lines *a* and *b*), a difference that contrasts with these species' similarity in maximum appetite (Fig. 1, lines *a* and *b*). While the reason for the discrepancy in these estimates is not immediatley evident, the close phylogenetic relationship between coho and brown trout, their ecological similarity, and comparable maximum appetites suggest that it could well be an artifact of methodological differences. Elliott (1975b) obtained the sustained feeding rate of brown trout indirectly by multiplying maximum appetite (Elliott 1975a; following 72 h of starvation) by the expected number of meals in a day (Elliott 1975b). Because he noted little variation in meal size over four consecutive meals, Elliott's conclusion that all meal sizes would equal the maximum seems valid. However, Elliott (1975b) may not have continued satiation feeding for a sufficient number of meals to observe the decline in feeding rate obtained here. For coho, this decline became most evident after day 1.

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Growth Inhibition, Gluconeogenesis, and Morphometric Studies of the Pituitary and Interrenal Cells of Acid-Stressed Brook Trout (*Salvelinus fontinalis*)

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Somatic growth was stunted in yearling brook trout (*Salvelinus fontinalis*) maintained for up to 73 d in pH 4.5. Plasma cortisol level increased in pulses after 1.5 h (from 10 to 82.3 ng·mL^{-1}) on days 4 (46 ng·mL^{-1}) and 15–30 (20–25 ng·mL^{-1}) of acid treatment. Interrenal nuclear hypertrophy and cell hyperplasia occurred respectively on days 4 and 15. Electron microscopic observations revealed that the somatotropes and thyrotropes were atrophic for about half of the experimental period whereas the corticotropes displayed sustained hypertrophy. Plasma glucose and amino acid levels rose simultaneously by 300–600 and 130%, respectively, from day 4 of acid exposure. Hepatic L-alanine aminotransferase activity increased on day 30 just as plasma protein concentration began to decline. When adrenalin and cortisol acetate were administered to trout maintained in neutral pH to raise circulating cortisol levels from 10–20 to 1876 ng·mL^{-1}, blood glucose remained normal up to 6 d after treatment. These results suggested that acid stress suppressed somatotropin, thyrotropin, and indirectly thyroid hormone secretion but stimulated the pituitary–interrenal axis, leading to inhibition of protein synthesis and accumulation of amino acids in the circulation. The elevation of blood sugar level was caused by some as yet unidentified factors.

La croissance somatique a été retardée chez l'ombre de fontaine (*Salvelinus fontinalis*) de moins de un an placé pendant une période maximale de 73 d dans une eau de pH 4,5. On a observé des poussées du taux de cortisol plasmatique après 1,5 h (10 à 82,3 ng·mL^{-1}), le quatrième jour (46 ng·mL^{-1}) et du 15^{e} au 30^{e} jour (20–25 ng/mL^{-1}) du traitement en milieu acide. On a observé une hypertrophie nucléaire interrénale et une hyperplasie cellulaire les 4^{e} et 15^{e} jours. Des examens au microscope électronique ont montré que les somatotropes et les thyréotropes étaient atrophiques pendant près de la moitié de la période expérimentale tandis qu'on a relevé une hypertrophie soutenue des corticotropes. Les taux de glucose plasmatique et d'acides aminés ont augmenté simultanément de 300 à 600 % et de 130 % respectivement à partir du 4^{e} jour d'exposition. L'activité de l'aminotransférase de la L-alanine hépatique a augmenté le 30^{e} jour juste au moment où la concentration des protéines plasmatiques commençait à décliner. Lorsque l'on a administré de l'adrénaline et de l'acétate de cortisol à l'omble placé dans une eau à pH neutre afin de faire passer le taux de cortisol en circulation de 10–20 à 1 876 ng·mL^{-1}, le glucose sanguin est demeuré normal jusqu'à 6 d après le traitement. Il semble, d'après ces résultats, qu'une exposition à un milieu acide supprime la sécrétion de somatotrophine, de thyrotrophine et indirectement de l'hormone thyroïdienne, mais qu'elle stimule l'axe hypophyso–interrénal, entraînant une inhibition de la synthèse des protéines et l'accumulation d'acides aminés dans la circulation. L'élévation de la glycémie était causée par certains facteurs non encore identifiés.

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Growth inhibition appears to be a common response in some fish species to acid stress. Such inhibition is usually reported as an actual decrease in body weight (Beamish et al. 1975) or a reduction in the rate of weight increase (Menendez 1976; Haines 1981; Tam and Payson 1986). Only in the white sucker (*Catostomus commersoni*) was it clearly documented that weight loss was associated with the absence of linear growth (Beamish et al. 1975). Inhibition of linear growth suggests a lack of somatotropin, and weight loss implies abnormalities in nutrient metabolism.

The effects of acid stress on the secretion of pituitary hormones in fishes have not been extensively investigated. Acid stress has been shown to influence the prolactin cells and the pars intermedia PAS-positive (PIPAS) cells. In tilapia (*Oreochromis mossambicus*), acid exposure resulted in a marked increase in the secretion of prolactin as assessed by morphometric and biochemical methods but had no noticeable effects on the PIPAS cells (Wendelaar Bonga et al. 1984a, 1984b). Conversely, simlar morphometric studies of the goldfish (*Carassius auratus*) pituitary revealed no observable influences of acid exposure on the cytological appearance of the prolactin cells but a notable increase in the secretory activity of the PIPAS cells (Wendelaar Bonga et al. 1984b). Investigations of the effects of acid exposure on the activity of pituitary cells which secrete hormones having well-established roles in the regulation of fish growth and metabolism — particularly somatotropin (GH), adrenocorticotropin (ACTH), thyrotropin (TSH) — have not as yet been reported.

Although increases in plasma cortisol levels in acid-stressed fish have been observed (Mudge et al. 1977; Brown et al. 1984), there is a discrepancy in the timing of corticosteroid increases that makes it difficult to correlate cortisol fluctuations with other physiological changes taking place during this critical period. Increases in blood glucose levels of acid-stressed rainbow trout (*Salmo gairdneri*) (Brown et al. 1984; Barton et al. 1985) and brook trout (*Salvelinus fontinalis*) (Tam and Payson 1986) have been observed. However, it is not known whether the extra blood sugar was derived from the carbohydrate, fat, or protein reserves of the body as the result of the high circulating levels of cortisol.

The purpose of this study was to investigate perturbations in growth, changes in the activity of the interrenal tissue, and a number of indices of carbohydrate metabolism of acid-treated brook trout and to correlate these biochemical changes with ultrastructural and morphometric investigations of the somatotropes, corticotropes, and thyrotropes. To investigate possible seasonal and reproductive effects, two similar experiments were performed, one under winter and the other under summer conditions. The winter experiment was terminated on day 52 and the summer experiment was terminated on day 73 of acid exposure. These periods were selected because previous experiments on brook trout demonstrated that growth retardation was most pronounced during the first 2 mo following acid exposure after which time the fish exhibited signs of acclimation (Tam et al. 1986a). In a third experiment, trout were injected with adrenalin and cortisol and maintained in water of neutral pH to test the effects of these hormones on plasma glucose concentration.

Materials and Methods

Fish Maintenance and Sample Collection

In experiment 1 (winter experiment), 12-mo-old sexually immature brook trout were purchased from Goossens Trout Farm (Otterville, Ont.) on January 9, 1986. After a month long acclimation to laboratory conditions, exposure to pH 4.5 (sulphuric acid) began on February 9 (day 0) and the experiment was terminated on April 2 (day 52). In experiment 2 (summer experiment), 16-mo-old brook trout were again purchased from the same commercial source on April 10, 1986. Water for the experimental fish was acidified to pH 4.5 on June 15 (day 0) and the experiment was completed on August 27 (day 73). Experiment 2 was therefore performed during the initial 2 mo of the first reproductive season of the fish (Tam et al. 1986b).

The chemical characteristics of the water (London, Ont., city water) used have been published in another report (Fryer et al. 1988) which shows that principal characteristics of the water such as alkalinity (82.5 ± 2.3 (6) $mg \cdot L^{-1}$ as $CaCO_3$), conductivity (244.2 ± 7.1 (3) $\mu mho \cdot cm^{-1}$), and major ions (sodium, 8.98 ± 0.02 (10) $mg \cdot L^{-1}$; sulphates, 32.1 ± 9.1 (4) $mg \cdot L^{-1}$) remained stable throughout the year. The methods of water dechlorination and temperature control, photoperiod regulation to mimic the natural photoperiod, and fish maintenance have also been detailed in a previous publication (Tam and Payson 1986). A number of improvements in fish maintenance were introduced. The fish were maintained in larger, fibreglass-lined cement tanks (2.54 × 0.61 × 0.48–0.55 m deep). The water for the acid-treated fish was acidified and chilled to the same temperature as the holding tanks 24 h before use. The carbon dioxide generated in the water by the addition of sulphuric acid was removed as much as possible by aeration and mechanical agitation. The fish were fed once daily at 1600–1700 to near satiation with a weighed amount of commercial trout food (Martin Feed Mills Limited, Elmira, Ont.) containing 40% protein, 10% fat, 3% fibre, and an array of vitamins. Fish were usually sacrificed between 0900 and 1600 with a sharp blow to the head within seconds of capture to avoid handling stress. Body weight and fork length were recorded. Intraperitoneal fat, plasma, and liver samples were collected in manners described before (Tam et al. 1987). Usually 12 fish from each control and acid group were sacrificed on each day of sample collection, giving an average of 6 male and female fish per group. However, the range for the number of observations was 2–15. The low numbers occurred on days 38 and 52 of experiment 1 and days 8 and 72 of experiment 2 when the fish killed were predominantly of one sex.

Injection Protocol

Cortisol acetate (Sigma Chemical Company, St. Louis, MO) was suspended in Tween 80 (10 $mg \cdot mL^{-1}$). Adrenalin injections (1 $mg \cdot mL^{-1}$) were purchased from Allen and Hanburys (Toronto, Ont.). In experiment 3, the experimental fish were either treated once with injections of 0.5 mg of cortisol acetate and 0.1 mg of adrenalin or twice, with the second treatment given 4 d later. Control fish were given 0.5 mL of Tween 80 each time. All injections were performed i.p. at 1000–1100 in June 1986. The mean weight of the fish was 327 ± 15 (59) g.

Biochemical Methods

The methods for the extraction and radioimmunoassay of plasma cortisol and the determination of plasma glucose and liver glycogen have been given elsewhere (Tam et al. 1987). After the blood proteins had been removed by precipitation with sulphosalicylic acid, free plasma amino acids in brook trout plasma were qualitatively and quantitatively analyzed with a Beckman model 119CL single-column amino acid analyzer

(Beckman Instruments Inc., Palo Alto, CA). Plasma-free amino acid content was qualitatively almost the same as that of the Beckman System 6300 amino acid standard. Subsequent determinations were performed by adding sodium bicarbonate and 2,4,6-trinitrobenzene sulphonic acid to the supernatant of the plasma and sulphosalicylic acid mixture. After incubation at 40°C for 2 h, the absorbance of the condensation products of the sulphonic and amino acids was measured at 420 nm. The amount of amino acid in the sample was calculated from a standardization curve constructed with known amounts of the Beckman System 6300 amino acid standard. Plasma protein concentration was assayed by the Lowry method (Lowry et al. 1951). After the liver samples were collected, they were stored at −80° for no more than a few days before use. The liver sample was homogenized in Tris-EDTA buffer (pH 7.4). The homogenate was filtered through two layers of cheese cloth and then made up to 5 mL with buffer. The activity of L-alanine aminotransferase in a known aliquot of the homogenate was assayed by using the Sigma alanine amino transferase reagent (Sigma Chemical Co.). One unit of activity is defined as the amount of enzyme that produces sufficient pyruvate to oxidise 1 μmol NADH·min^{-1}.

Interrenal Cell Cytological Techniques

The head kidney was dissected out and fixed in 4CF-1G diagnostic fluid (McDowell and Trump 1976). Most of the interrenal tissue was found at the base of the two short limbs of the T-shaped head kidney. After embedding in paraffin wax, 60 cross-sections (6 μm) were cut from these regions and stained with Gomori's trichrome and haematoxylin. Interrenal tissue was studied histologically with a light microscope and morphometric measurements were made with a Bioquant II computer-assisted morphometric system (R. & M. Biometrics Inc., Nashville, TN). Mean interrenal nuclear areas for each stage of the experiment were calculated from measurements of 50–150 of the largest nuclei. Mean interrenal cell areas were calculated as the quotients of 6–12 areas of interrenal tissue and the number of nuclei enclosed within these areas. Total interrenal tissue within each head kidney was estimated by calculating the area occupied by the maximum amount of interrenal tissue located within 10 000 μm² of head kidney.

Pituitary Electron Microscopy

For ultrastructural studies of the endocrine cells of the pituitary gland, pituitaries were dissected and fixed by immersion in 2% paraformaldehyde – 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, containing 0.05% calcium chloride at 4°C. After 1–2 h, the fixative was replaced with fresh fixative and the pituitaries were maintained at 4°C over night. The next day the fixative was discarded and replaced with 0.1 M cacodylate buffer, pH 7.4, containing 0.05% calcium chloride and 7% dextrose and the pituitaries stored at 4°C. Pituitaries were cut in half in the sagittal plane and each hemipituitary postfixed for 2 h with 1% osmium tetroxide in 0.1 M cacodylate buffer containing 7% dextrose. The hemipituitaries were dehydrated with a graded series of alcohols and propylene oxide and then embedded in araldite. For orientation purposes, serial thick (1 μm) sections of each hemipituitary were cut with an LKB Ultratome III and stained with methylene blue and asure II. Following the identification and localization of the endocrine cells in the thick section, the block was trimmed and thin sections cut and stained with uranyl acetate and lead citrate. Sections were examined with a Philips EM 300 electron microscope. A carbon grating calibration grid (2160 lines·mm^{-1}) was used to standardize the magnification for each session in which electron micrographs were taken for morphometric analysis.

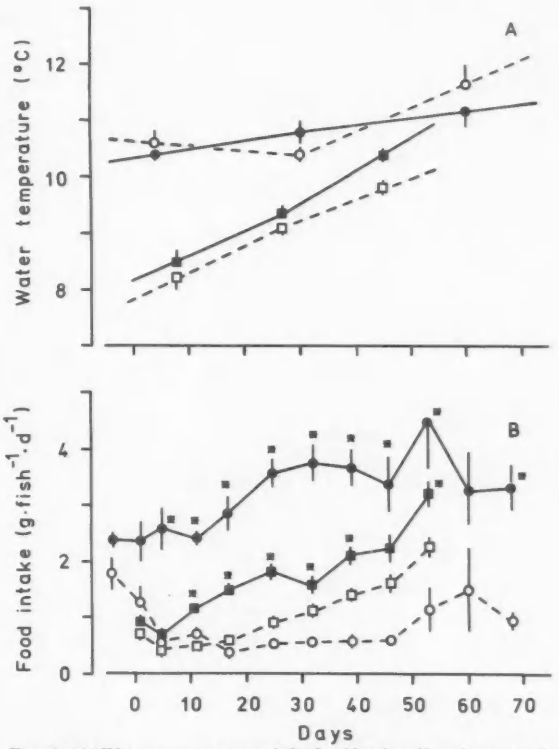


FIG. 1. (A) Water temperature and (B) food intake of brook trout maintained in neutral pH (■, experiment 1; ●, experiment 2) and pH 4.5 (□, experiment 1; ○, experiment 2). Asterisks indicate significant difference in *t*-test between means taken on the same day of the same experiment. Mean number of observations for temperature = 24 (range 18–31) and food intake = 7 d.

Pituitary Morphometry

For morphometric studies of the somatotropes, corticotropes, and thyrotropes, electron micrographs were printed at a final magnification of 3240. Total cell area and nuclear area were determined with a digitizing tablet connected to an IBAS I morphometric analyzer. Only cells containing nuclei were sampled. The cytoplasmic area was determined for each cell by subtracting the nuclear area from the cell area. For each cell type a minimum of 60 cells was measured from three to five female trout.

Statistical Treatment of Data

The results are expressed as mean ± SE (number of observations or fish in parentheses). The relationship between fork length and body weight and duration of experiment was estimated by regression analysis. Significant changes in the rate of increase in fork length or body weight between control and experimental groups were detected by comparing the slopes of the regression lines. Two-way analysis of variance (ANOVA) was used to test the effects of pH, duration of acid exposure,

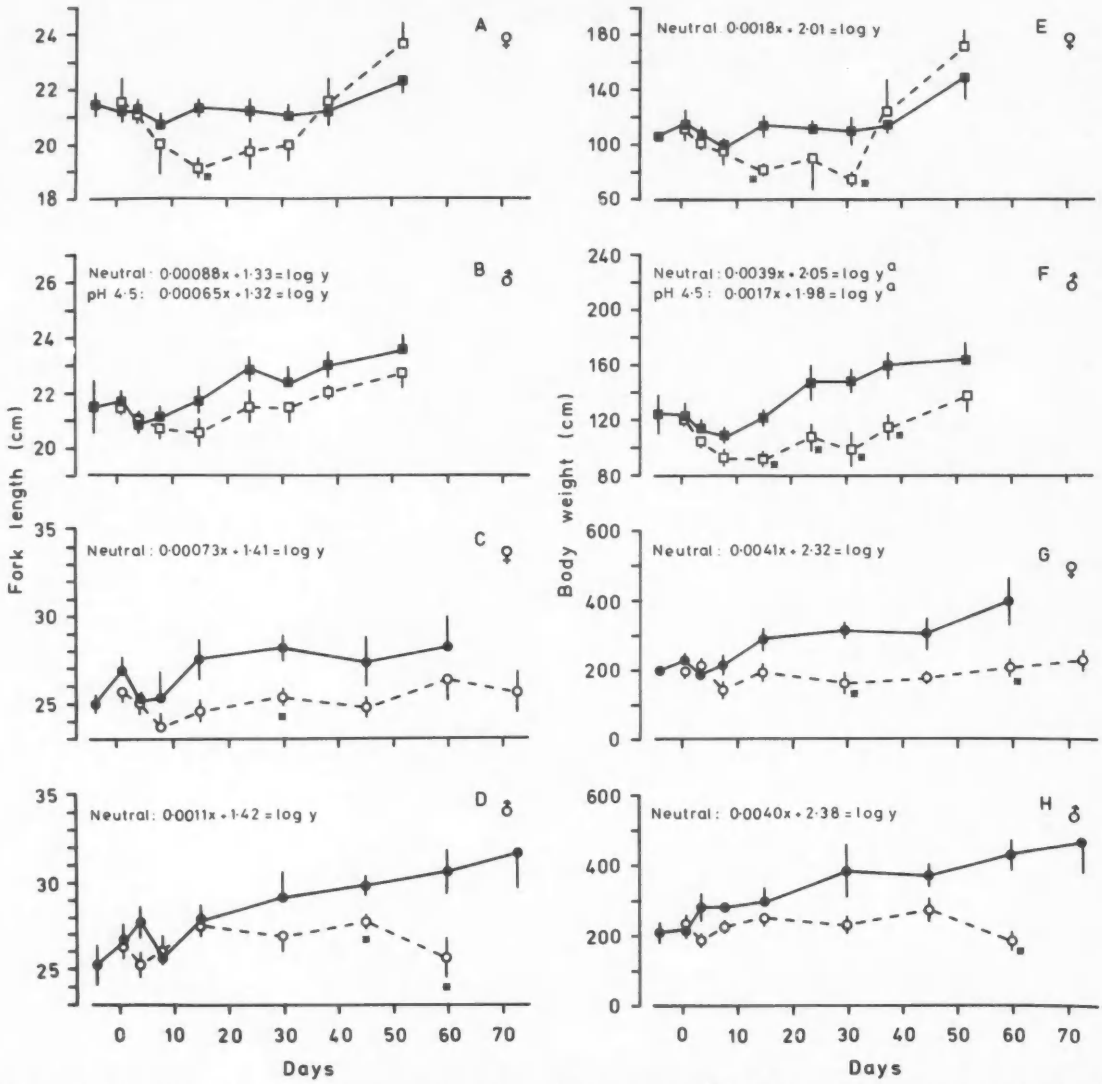

A ♀
Neutral: 0·00088x + 1·33 = log y
pH 4·5: 0·00065x + 1·32 = log y
B ♂
Neutral: 0·00073x + 1·41 = log y
C ♀
Neutral: 0·0011x + 1·42 = log y
D ♂
Neutral: 0·0018x + 2·01 = log y
E ♀
Neutral: 0·0039x + 2·05 = log y a
pH 4·5: 0·0017x + 1·98 = log y a
F ♂
Neutral: 0·0041x + 2·32 = log y
G ♀
Neutral: 0·0040x + 2·38 = log y
H ♂
Fork length (cm)
Body weight (cm)
Days
Days

FIG. 2. (A–D) Fork length and (E–H) body weight of brook trout maintained in neutral pH (■, experiment 1; ●, experiment 2) and pH 4.5 (□, experiment 1; ○, experiment 2). Only the regression lines with slopes significantly different from zero are given; *a* = significant difference in slopes. Asterisks indicate significant difference in *t*-test between means of experimental and control groups taken on the same day. Mean number of observations = 6 (range 2–15).

and the interaction of these factors on the condition factor (body weight × fork length $^{-3}$ × 100), relative peritoneal fat weight, plasma levels of glucose, free amino acids and cortisol, liver glycogen concentration, activities of hepatic L-alanine aminotransferase, and interrenal cell morphometry throughout the length of the experiments. Student's *t*-test was also used to test for significant differences between the means of corresponding experimental and control groups taken on any particular sampling date.

For morphometric studies of the somatotropes, corticotropes, and thyrotropes, statistical differences between groups were assessed using Student's *t*-test for unpaired observations. When the data were not normally distributed, we used the non-parametric Mann–Whitney *U*-test. In all cases the data were tested for significance at the 5% level.

Results

Water Temperature, pH, and Food Consumption

The pH of the water in the acidified tanks (experiment 1, pH 4.55 ± 0.01, n = 88; experiment 2, pH 4.54 ± 0.07, n = 96) remained very constant and close to the target value of pH 4.5. The pH of the water for the control groups was 7.26 ± 0.02 (198). Water temperature rose about 2° in experiment 1 and about 1° for the duration of experiment 2 (Fig. 1A). In both experiments, food consumption by the acid-treated fish was significantly reduced (Fig. 1B).

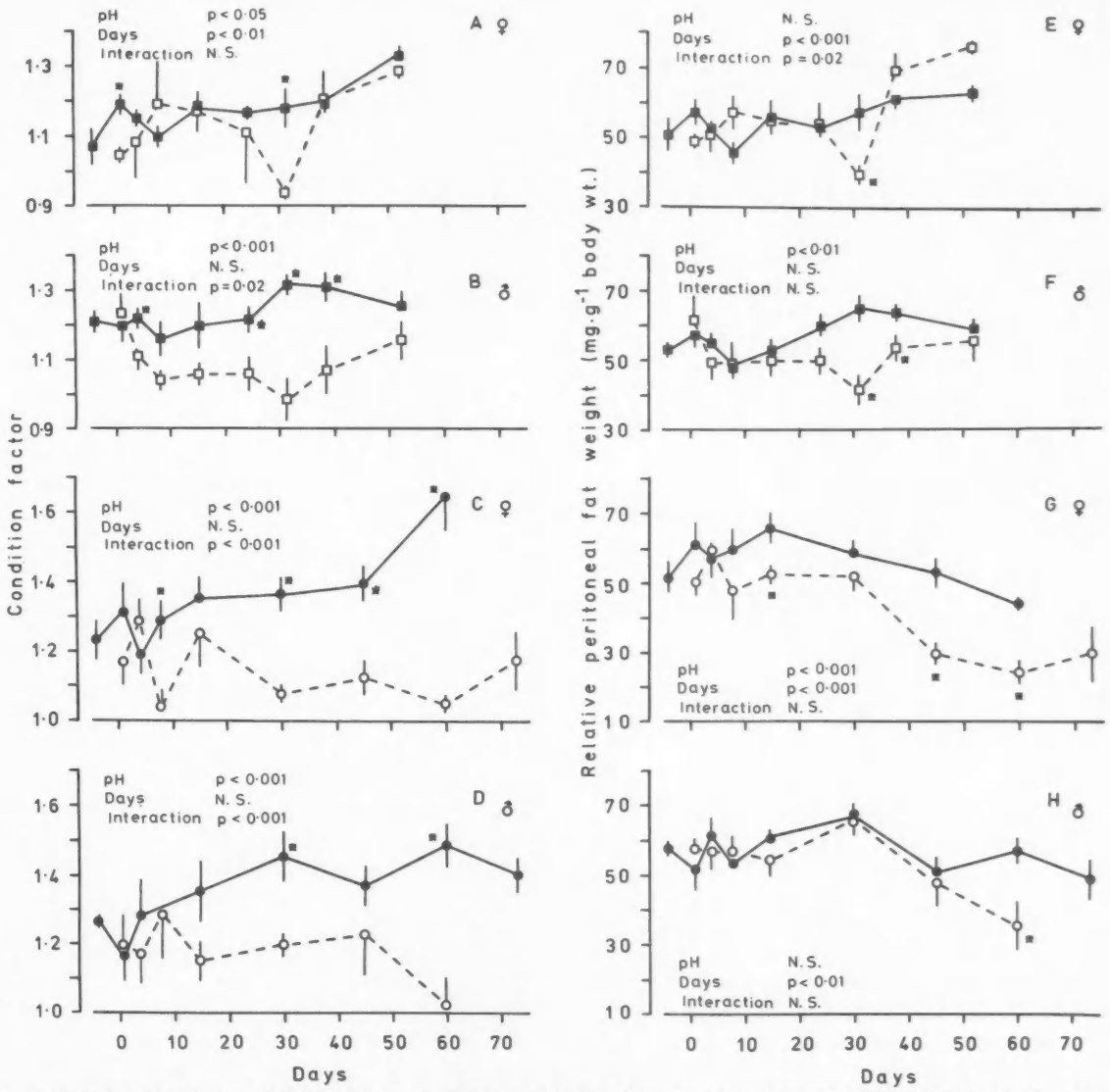


FIG. 3. (A–D) Condition factor and (E–H) relative peritoneal fat weight of brook trout maintained in neutral pH and pH 4.5. Results of two-way ANOVA are also given; N.S. = not significant. Numbers of observations, their range, and meanings of symbols are as defined in Fig. 2.

Physical Conditions of the Fish

Although no significant increase in fork length was evident among the females of experiment 1 (Fig. 2A), a small but significant increase in body weight was observed in the control fish (Fig. 2E). Significant linear growth (Fig. 2B) and increase in body weight (Fig. 2F) were observed in all the males. However, the rate of weight increase in the acid-treated fish was significantly lower than that of control fish. In experiment 2, fork length increased by 13–26% and body weight by as much as 101–123% in control fish (Fig. 2C, 2D, 2G, and 2H). Neither linear growth nor body weight increase was observed in the acid-treated fish. Two-way ANOVA revealed that low pH significantly decreased condition factor in all acid-treated groups (Fig. 3A–3D). Significant interaction between pH and duration of acid exposure was observed in three of the four acid-treated groups, indicating that within these groups, condition factor declined steadily as the experiment progressed. However, in the same three groups of acid-stressed fish, there was no significant interaction between pH and duration of experiment on intraperitoneal fat concentration (Fig. 3E–3H). Therefore, there was no progressive effect of acid on fat weight. Indeed, *t*-tests revealed that significant reduction in fat concentration occurred almost only after day 30.

Plasma Levels of Glucose, Amino Acids, and Proteins

Despite seasonal differences in temperature and photoperiod, plasma glucose levels of control fish were constant at about 65–72 mg·dL^{-1} in both experiments (Fig. 4A and 4B). Plasma glu-

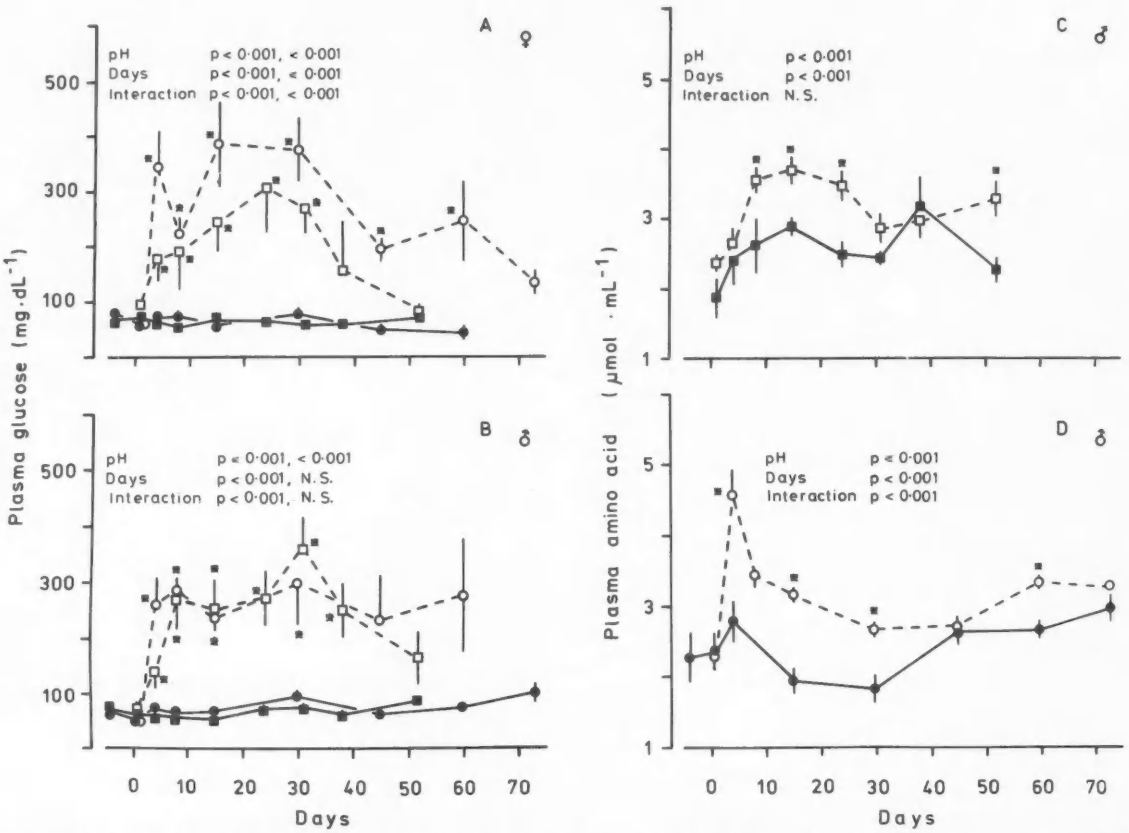


FIG. 4. (A and B) Plasma glucose concentration and (C and D) free amino acid level of brook trout maintained in neutral pH and pH 4.5. Mean number of observations for glucose = 6 (range 2–14) and amino acid = 6 (range 3–10). Meanings of symbols and abbreviations are as defined in Fig. 3.

cose levels of acid-exposed fish increased significantly from day 4 to 200–400 mg·dL^{-1}. In experiment 1, it tended to return to control level after about a month, but this tendency did not occur until later on in the second experiment. Plasma amino acid concentrations, which were measured only for the males (Fig. 4C and 4D), increased significantly in the acid-stressed fish on day 4 of the experiment. This increase was more pronounced during the first 30 d. Plasma protein concentration remained constant among the control fish (Fig. 5A). In the acid-treated fish, it increased significantly between days 1 and 4 (*t*-test, $p < 0.02$) and then decreased progressively throughout the rest of the experiment.

Hepatic Alanine Aminotransferase Activity and Glycogen Concentration

The activity of hepatic alanine aminotransferase was assayed only in the males of experiment 2 (Fig. 5B). Significant increase in the activity of this enzyme was observed after 30 d of acid exposure. Liver glycogen concentrations varied between 32 and 75 mg·g wet liver weight^{-1} and no significant change could be ascribed to acid treatment.

Plasma Cortisol Levels

Plasma cortisol levels from male and female fish were pooled, as no significant difference due to sex was detected (Fig. 6A, 6B, and 6C). Plasma cortisol levels within the first 24 h of acid exposure were assayed only in experiment 2 (Fig. 6C). In control fish, plasma cortisol levels varied between 10 and 20 ng·mL^{-1}, but those of the acid-treated fish rose to 82.3 ± 23.4 (5) ng·mL^{-1} at 1.5 h after acid exposure before returning to control levels at 5 h. Within the first 24 h, plasma glucose increased transiently at 3 h after acid treatment (Fig. 6D). After day 1, plasma cortisol levels displayed a similar pattern in experiments 1 and 2 (Fig. 6A and 6B). Plasma cortisol level rose to another peak on day 4. It declined between days 8 and 15 and then tended to rise somewhat again between days 15 and 30.

Interrenal Cell Histology and Morphometry

The diffused masses of interrenal tissue were found bordering the larger blood sinusoids of the head kidney. The cells were columnar to polygonal. The cytoplasm was acidophilic and in the control fish it was finely granulated. The nuclei in the control fish were round to avoid, heterochromatic, and their nucleoli were indistinct. In the acid-treated fish, the cytoplasm was more vacuolated and the nuclei were large with diffuse chromatin. Their nucleoli were distinct and centrally located. Acid treatment resulted in significant increases in interrenal nuclear area

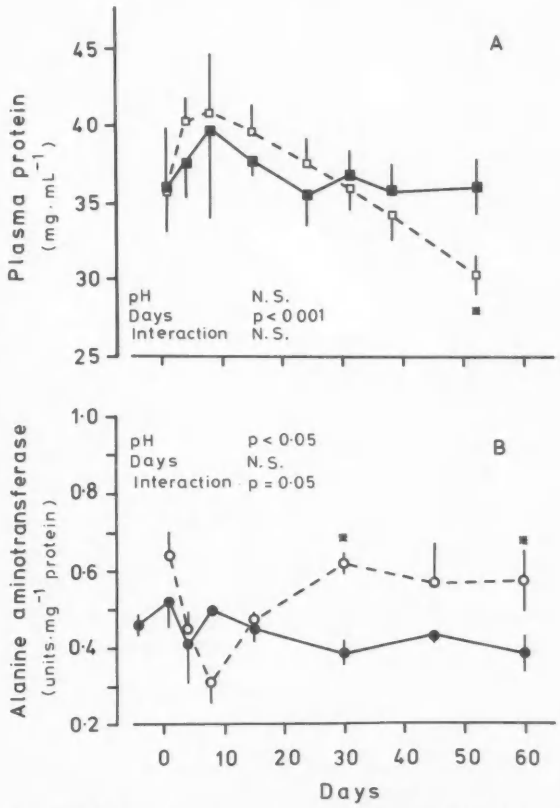


FIG. 5. (A) Total plasma protein concentration and (B) activity of hepatic L-alanine aminotransferase in male brook trout maintained in neutral pH and pH 4.5. Mean number of observations for protein concentration = 7 (range 3–10) and aminotransferase = 4 (range 2–6). Meanings of symbols and abbreviations are as defined in Fig. 3.

between days 4 and 45 with a peak occurring on day 4 (Fig. 7A). Except on day 15, interrenal cell size remained unchanged in acid-treated trout (Fig. 7B). However, much larger masses of interrenal tissue were observed along the blood sinusoids after day 15, suggesting that interrenal hyperplasia had occurred. Estimation of total interrenal tissue by measuring interrenal cross-sectional area also revealed a significant increase (two-way ANOVA) that could be attributed to low pH (Fig. 7C). Although these histological parameters were not measured in experiment 1, microscopic examination of the interrenal tissue confirmed a similar pattern of changes.

Pituitary Cytology

The organization of the pituitary is generally similar to the arrangement of the pituitary in other salmonid fishes (Cook and van Overbeeke 1972; J. N. Fryer et al., unpubl. data). The rostral pars distalis consists of prominent follicles composed exclusively of prolactin cells with corticotropes appearing caudally. The proximal pars distalis contains thyrotropes, somatotropes, and gonadotropes. The neurointermediate lobe contains melanotropes dispersed along the arborizations of the neurohypophysis. A striking feature of the brook trout pituitary is the large number of nonsecretory stellate cells which invest the endocrine cells and frequently extend into, and partition off, small areas of cytoplasm around the periphery of the endocrine cell (Fig. 8 and 10). Accordingly, for purposes of morphometry, an endocrine cell was considered to consist of a nucelus and contiguous cytoplasm excluding any peripheral cytoplasmic partitions. Such cytoplasmic partitions could not be determined as belonging to an individual endocrine cell.

Somatotropes

The somatotropes are located in the central region of the proximal pars distalis subjacent to the thyrotropes. The somatotropes are arrayed in clusters surrounded by thyrotropes and gonadotropes and are often invested with a layer of the stellate cell cytoplasm. The somatotropes are small cells and are readily distinguished by the large size of the nucleus with a sparse cytoplasm. In control trout (Fig. 8A) the cytoplasm of the somatotropes contained abundant spherical electron-dense secretory granules, and the rough endoplasmic reticulum was restricted to a few narrow cisternae in the paranuclear region. Mitochondria and Golgi bodies were routinely observed.

Acid exposure resulted in no readily discernible changes in the cytological appearance of the nuclei or in the size of the somatotropes (Fig. 8B). Indeed, the somatotropes of acid-exposed trout were virtually indistinguishable in appearance from the somatotropes of control trout with the exception that by day 8 and subsequent sampling periods following acid exposure and rough endoplasmic reticulum was not as conspicuous in the cytoplasm of the acid-exposed trout.

Morphometric analysis of the somatotropes, however, revealed a progressive decrease in cell area and nuclear area and in cytoplasmic area which attained statistical significance on day 8 and on day 15, respectively, following acid exposure (Fig. 9A and 9D). The decrease in cell area and nuclear area was maintained at subsequent sampling periods. These morphometric data indicate that acid exposure results, after an initial lag period of about 1 wk, in an atrophy and a sustained reduction in the secretory activity of the somatotropes.

Corticotropes

The corticotropes are distributed in a narrow band two to four cells wide immediately adjacent to the neurohypophysis, caudal to the prolactin cells. The corticotropes are usually wrapped in a layer of stellate cell cytoplasm. In control trout (Fig. 10A), the nuclei of the corticotropes were prominent and the cytoplasm contained numerous "haloed" secretory granules with cores of irregular shape and variable electron density. The rough endoplasmic reticulum was well developed and appeared in the cytoplasm in aggregations of dense arrays of parallel cisternae. Golgi bodies and mitochondria were readily observed.

Acid exposure resulted in striking alterations in the appearance of the corticotropes readily observable on the first day following acidification and which were consistently noted at subsequent sampling periods (Fig. 10B). The corticotropes displayed a marked hypertrophy and the nuclei appeared larger. The rough endosplasmic reticulum was more prevalent and occupied extensive regions of the cytoplasm. Golgi bodies were more frequently observed than in the corticotropes of control trout. The corticotropes displayed the morphological correlates of increased secretory activity.

Morphometric analysis of the corticotropes following acid exposure revealed significant increases in cell area, nuclear area, and cytoplasmic area on day 1 following water acidifi-

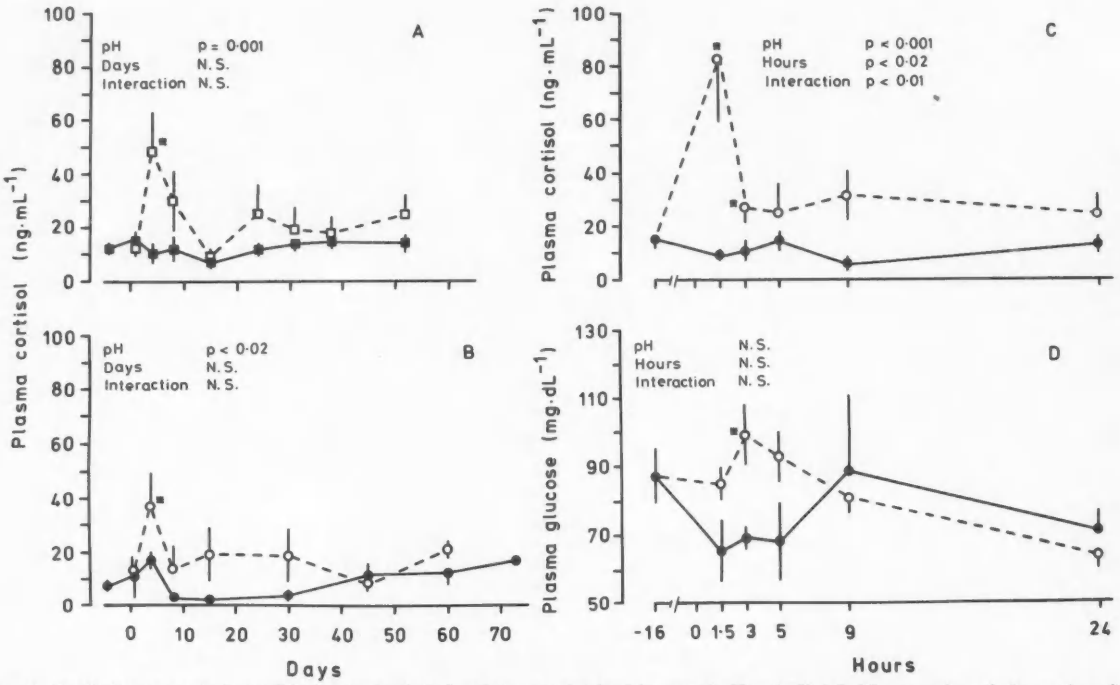


FIG. 6. (A–C) Plasma cortisol and (D) glucose levels in brook trout maintained in neutral pH and pH 4.5. Mean number of observations for cortisol = 6 (range 2–11) and glucose = 5 (range 4–7). Meanings of symbols and abbreviations are as defined in Fig. 3.

cation. These increases reached a maximum on day 15 and remained significantly higher than the corresponding cellular parameters of the control fish throughout the sampling period (Fig. 9B and 9E). These data indicate that acid exposure resulted in a hypertrophy and a sustained activation of the secretory activity of the corticotropes.

Thyrotropes

The thyrotropes are located in the central region of the proximal pars distalis bordering the neurohypophysis adjacent to the corticotropes. In control trout (Fig. 11A), the thyrotropes were large cells with a prominent nucleus. The cytoplasm contained numerous secretory granules which were heterogeneous in size and less electron dense than those of the somatotropes. Golgi bodies and mitochondria were apparent. Many of the thyrotropes contained large lipid droplets.

Acid exposure resulted in no visibly distinguishable changes in the cytological appearance of the thyrotropes (Fig. 11B). However, morphometric analysis revealed a significant decrease in nuclear area on day 4 following acid exposure which was maintained at subsequent sampling periods (Fig. 9C and 9F). Thyrotrope cell area decreased transiently and was significantly lower than control values on days 15 and 24 following acidification before returning to the control value on days 32 and 52. The cytoplasmic area of the thyrotropes displayed a pattern with time, which resembled the changes observed in cell area, but was less pronounced. A transient decrease in cytoplasmic area was observed which attained statistical significance only on day 24 following exposure to acid water. These morphometric data reveal that after an initial lag period of 2–3 d, acidification results in an atrophy of the thyrotropes which gradually recover but a sustained atrophy of their nuclei. These observations indicate that acid exposure results in a reduction in the secretory activity of the thyrotropes.

Effect of Adrenalin and Cortisol Acetate Injections on Plasma Glucose and Amino Acid Concentrations

In experiment 3, the results from male and female fish have again been pooled, as no distinction could be made between the sexes. The injection of Tween 80 alone was sufficient enough to significantly elevate plasma cortisol levels for several days (Table 1). Single injections of adrenalin and cortisol acetate raised the circulating levels of cortisol for 2–3 d, and two injections (days 0 and 4) maintained high plasma cortisol levels up to 6 d. However, no significant increase in blood sugar or amino acid levels could be attributed to the hormonal treatment.

Discussion

Our results probably reflected the direct and indirect effects of lowered environmental pH on fish. Although water temperature differed by a fraction of a degree between experimental and control fish, the temperatures of 8–12°C in experiments 1 and 2 lay well within the optimal temperature range for maximum activity in brook trout (cf. Power 1980). Reduced food consumption was one of the observed effects of acid stress in our experiments. Yet, physiological response in the acid-stressed brook trout was very different from that reported for fasting fishes, which typically showed normal plasma glucose levels and lowered serum amino acid concentrations for up to 6 mo without food (Lewis and Epple 1984; cf. Love 1980). However, the design of the present experiments cannot dem-

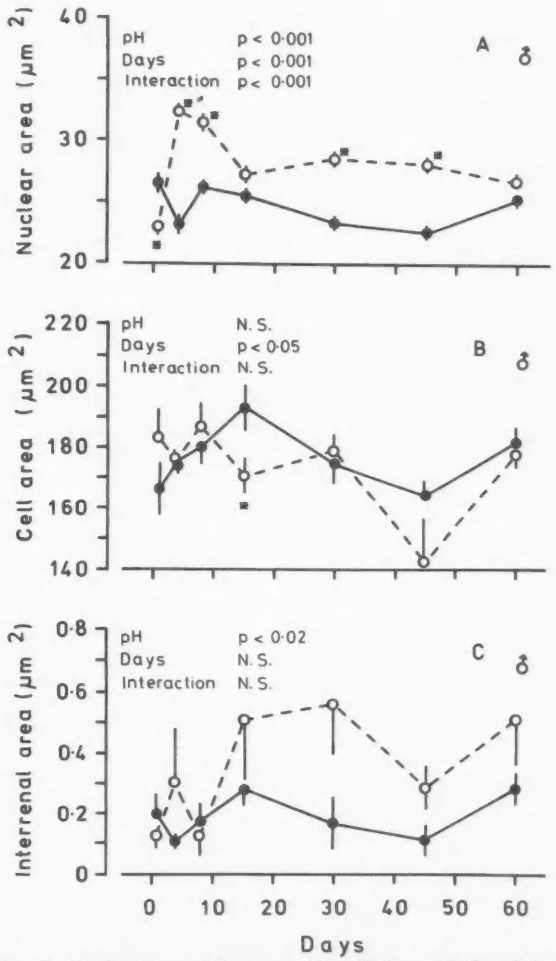


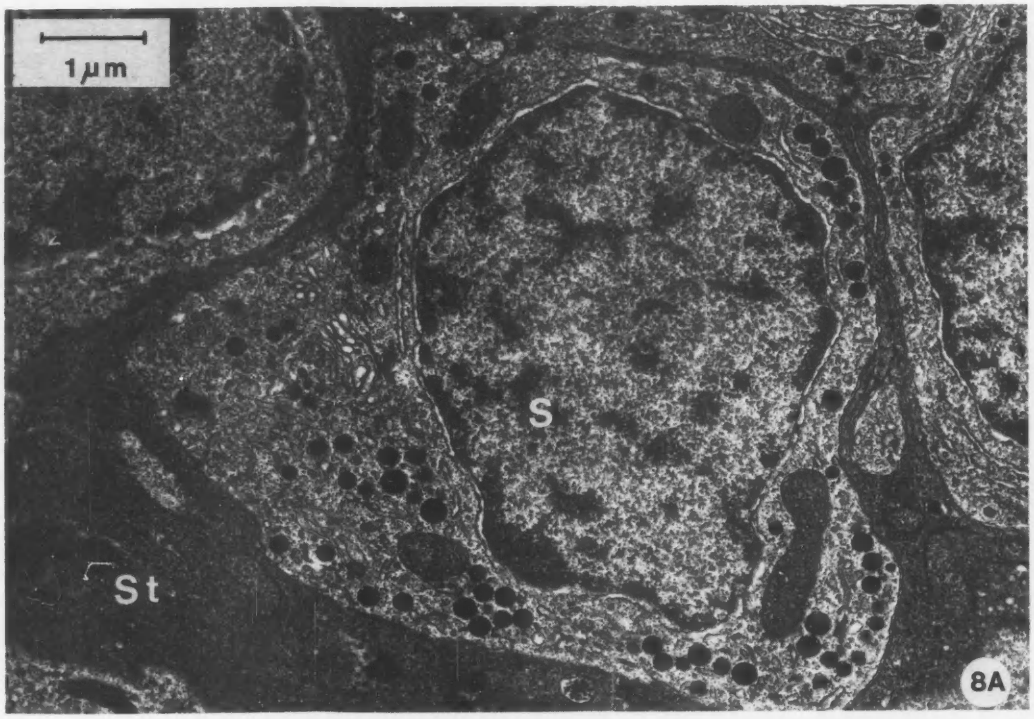
FIG. 7. (A) Interrenal nuclear area, (B) cell size, and (C) relative amounts of interrenal tissue in brook trout maintained in neutral pH and pH 4.5. Mean number of observations for nuclear size = 92 (range 30–156), cell size = 15 (range 5–25), and amount of tissue = 5 (range 4–6). Meanings of symbols and abbreviations are as defined in Fig. 3.

onstrate whether reduced food intake has constituted a secondary stress which modulates the direct effect of acid stress. Further investigation in this area is required.

Our results defined the charcteristics of growth inhibition in fish exposed to low environmental pH and provided some insight into the mechanisms by which growth was suppressed. Growth inhibition was more pronounced in the initial phase of acid exposure and in the summer months when normal growth occurred at a relatively faster rate. Seasonal and reproductive effects produced some quantitative but not qualitative differences. Growth inhibition was due to suppression of linear growth and abnormal endocrine and metabolic activities leading to a disproportionate decrease in body weight as revealed by the decrease in condition factor. Lack of linear growth may be due at least in part to a reduction in the secretory activity of the somatotropes which, once established after an initial lag period of about 1 wk, was maintained throughout the study. GH promotes increases in somatic growth of fishes and furthermore the growth-promoting effects of growth hormone are augmented by thyroid hormones (Higgs et al. 1982). Investigations in mammals have revealed that stress stimulates the release of somatostatin from the hypothalamus which inhibits the release of GH from the pituitary (Arimura et al. 1976). Although the presence of somatostatin in the teleost hypothalamus is well established (Olivereau et al. 1984) and somatostatin is known to inhibit the release of GH from the teleost pituitary (Fryer et al. 1979; Marchand et al. 1987), further work is required to clarify if the acid-stress-induced inhibition of somatotrope activity is mediated by somatostatin or some other hypothalamic or extrahypothalamic mechanism acting directly on the somatotropes. In this respect, the hyperglycemia and increased activity of the interrenal tissue observed in acid-exposed trout may be particularly significant, as GH secretion from the mammalian pituitary is inhibited by high circulating levels of glucose and glucocorticoids (Frohman 1981).

Increases in plasma cortisol levels in brook trout chronically exposed to low pH occurred in three successive waves with two sharp peaks detected respectively at 1.5 h and 4 d after acid exposure and a plateau between days 15 and 30. In trout, rapid increases in plasma cortisol concentration in the initial hours (1–3 h in brook trout; Mudge et al. 1977) and a slower but progressive rise between 8 and 20 d of acid treatment (in rainbow trout; Brown et al. 1984) have been observed in two experiments of shorter duration and with lower sampling frequency. The pulsatile mode of plasma cortisol increases observed in this study is probably a more accurate reflection of fish adapting to stress. However, plasma hormonal concentration is only a static assessment of the secretory activity of interrenal cells. Cytological studies of the response of the corticotrope–interrenal axis to acid stress offer another way of assessing the functional status of the interrenal cells. Acid exposure resulted in a concomitant hypertrophy and activation of the corticotropes and interrenal cells, lasting throughout the sampling period. The activation of the corticotropes represents a response to stress which is presumably mediated by the release of the neuropeptides of hypothalamic origin and which have corticotropin-releasing activity. Acid stress may result in the activation of neurons elaborating corticotropin-releasing factor, urotensin I, arginine vasotocin, or isotocin (Fryer and Lederis 1986). The possibility that other as yet unidentified factors of hypothalamic or extrahypothalamic origin may be involved in the activation of the corticotropes of acid-exposed fish should be entertained. Yet, the results of enhanced corticotrope activity could clearly be seen as increase in interrenal nuclear size (day 4) and hyperplasia (day 15 onward). Interrenal nuclear hypertrophy and cell hyperplasia have also been reported in rainbow trout subjected to 21 d of acid stress (Brown et al. 1984). Prolonged increases in plasma cortisol levels during confinement or acid exposure in coho salmon (*Oncorhynchus kisutch*) (Redding et al. 1984a) and rainbow trout (Brown et al. 1986) lead to an increased metabolic clearance rate of cortisol, masking the increased secretory rate of the interrenal cells. Therefore, the results in this report suggest that the first two peaks of plasma cortisol concentration were the result of ACTH stimulation and de novo synthesis. Interrenal hyperplasia occurred after day 15 of acid treatment in order to meet the continued demand for cortisol, but the concomitant increase in metabolic clearance rate attenuated any significant elevation in plasma coritsol concentration.

Acid exposure resulted in a sustained inhibition of the secretory activity of the thyrotropes after an initial lag period of 2–3 d. Investigations of the effects of acid exposure on thyroid



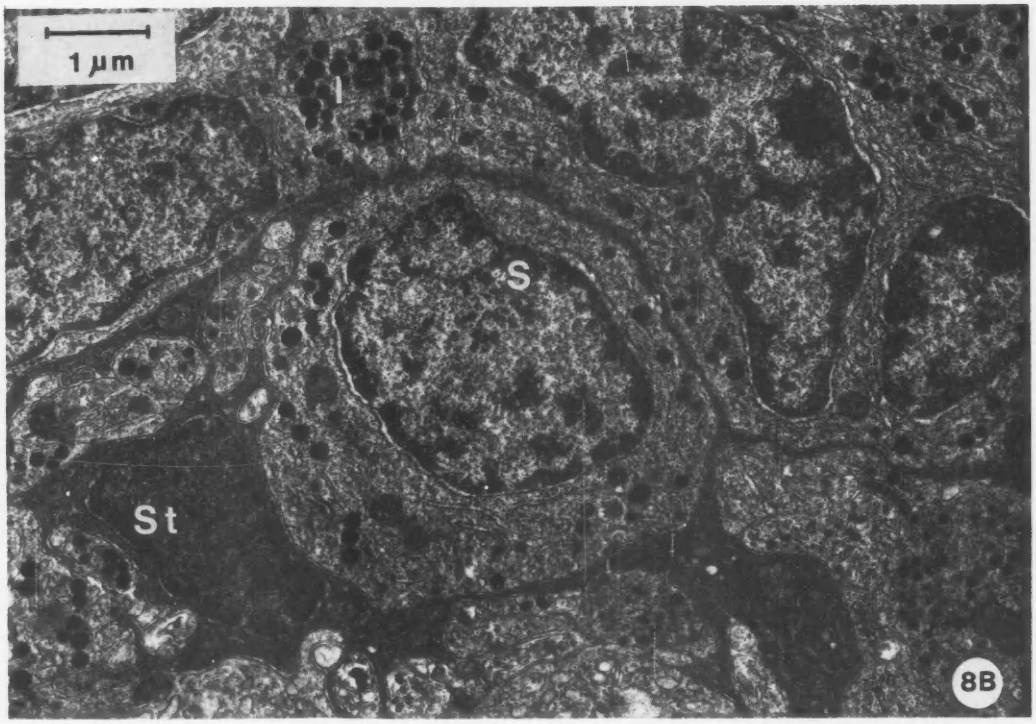


FIG. 8. Somatotropes of female brook trout (A) control and (B) exposed to acid water (pH 4.5) for 15 d. S, somatotrope; St, stellate cell.

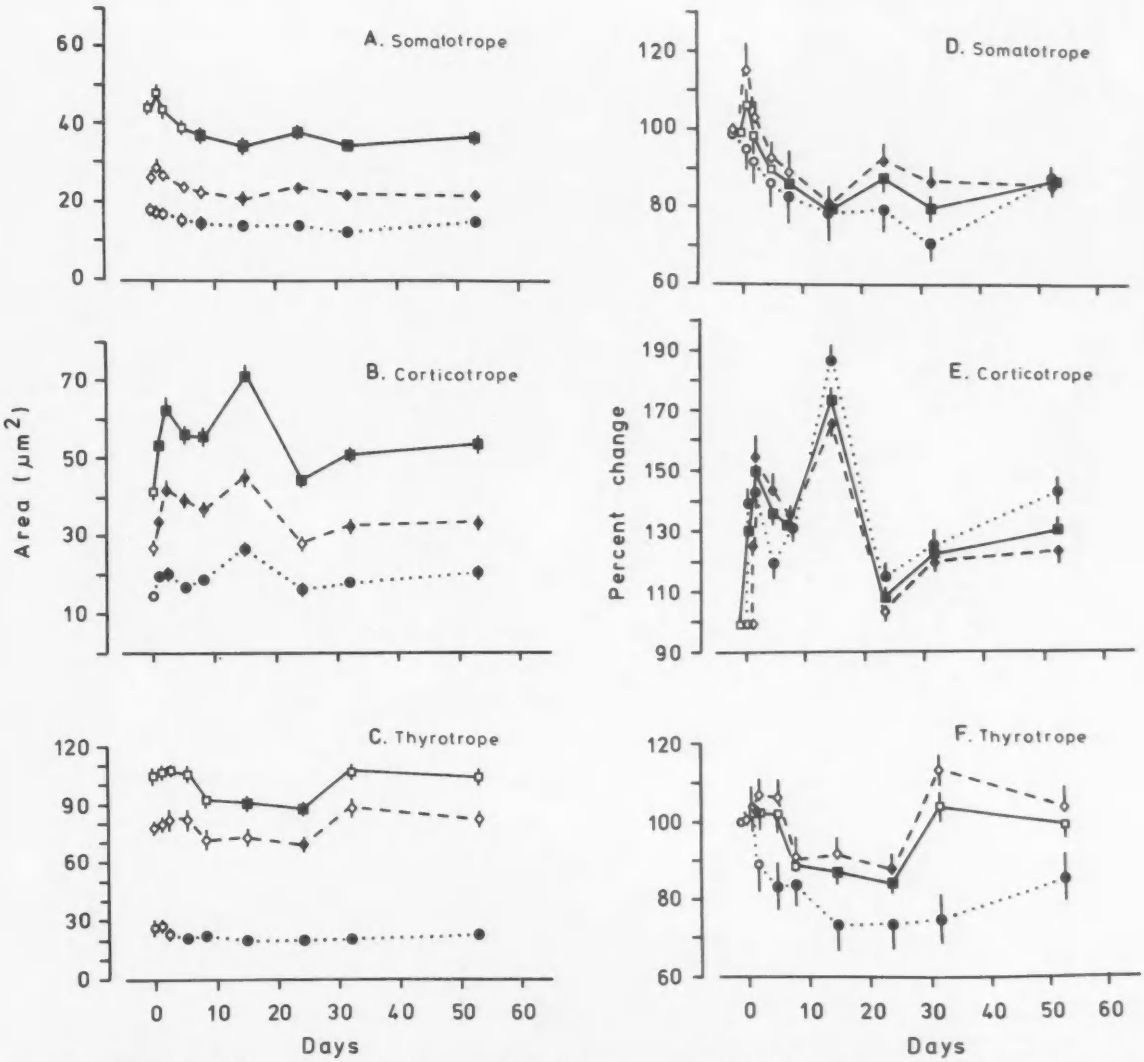


FIG. 9. Cell (■), nuclear (●), and cytoplasmic (◆) areas of (A and D) somatotropes, (B and E) corticotropes, (C and F) and thyrotropes in brook trout maintained in neutral pH (before day 0) and pH 4.5 (on and after day 0). A–C, absolute areas; D–F, results expressed as a percentage of the value found 4 d before acidification (−day 4). Solid symbols are significantly different from open symbols. Each point represents the mean of at least 60 observations.

physiology of the rainbow trout have shown that low pH tends to increase the ratio of plasma L-thyroxine (T4) to 3,5,3-triiodo-L-thyronine (T3) which was attributable to an elevation of T4 or a decrease in T3 depending upon the experiment (Brown et al. 1984). In addition, acid exposure was without effect on the histological appearance of the thyroid. However, in a subsequent study, plasma T4 or T3, or their ratio T4:T3, was not affected by acid exposure (Brown et al. 1986). The effects of acid exposure on circulating T4 and T3 remain unresolved but may be clarified by studies of the effects of acid exposure on plasma clearance rates of T3 and T4. In the present study, the factor(s) responsible for the inhibition of the activity of the thyrotropes cannot be determined. Acid stress may inhibit TSH secretion by hypothalamic neuroendocrine mediators, such as somatostatin, as has been shown in mammals subjected to stress (cf. Peterfreund and Vale 1983), or by factors exerting direct actions on the thyrotropes. In this respect, the sustained activation of the pituitary–interrenal axis is probably a contributing factor. In teleosts, corticosteroids generally inhibit the activity of the thyrotrope–thyroid axis. Cortisol administration results in thyrotrope atrophy (Leatherland and Lam 1971), thyroid epithelial cell atrophy (van Overbeeke and McBride 1971), and a decrease in circulating T3 and T4 (Redding et al. 1984b, 1986).

A decline in body weight or its rate of increase reflects alterations in metabolism which may be attributed to the endocrine changes induced by the acid treatment. Food consumption was decreased while at the same time energy was diverted from normal usage to meet the heightened costs of ionic and osmotic regulation. Sodium loss right at the onset of acid stress was observed in many fish species (Leivestad and Muniz 1976;

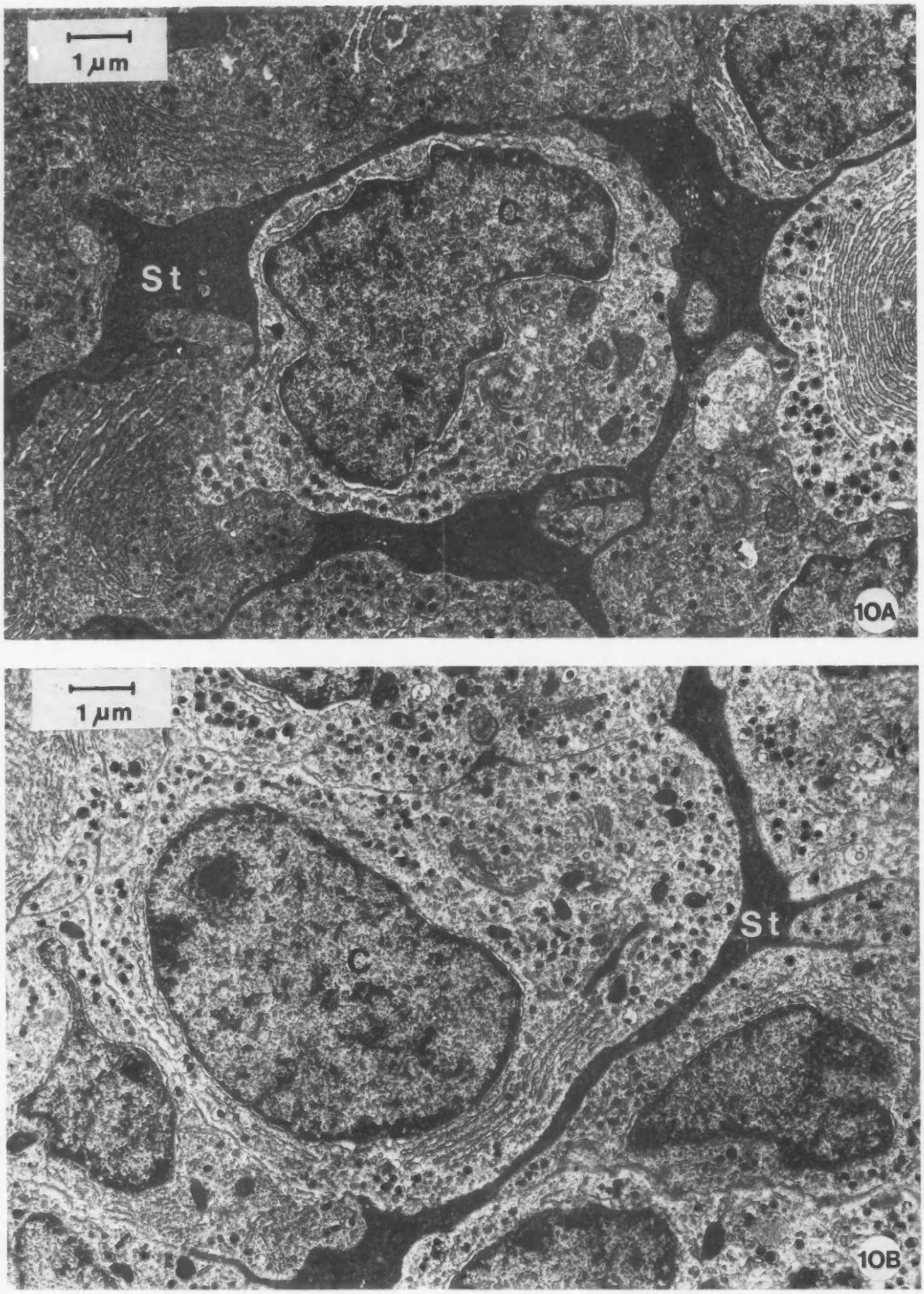


FIG. 10. Corticotropes of female brook trout (A) control and (B) exposed to acid water (pH 4.5) for 15 d. C, corticotrope; St, stellate cell.

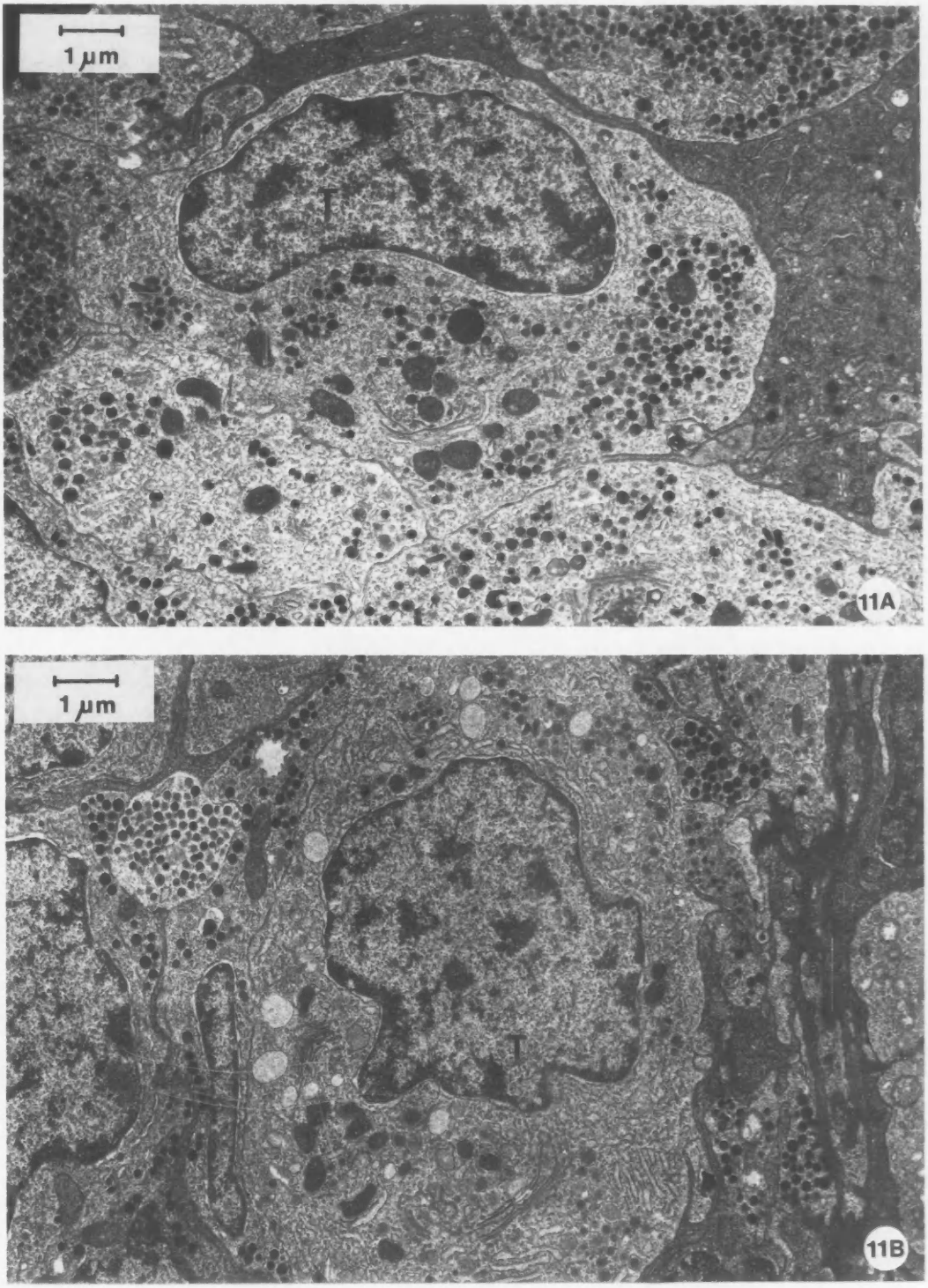


FIG. 11. Thyrotropes of female brook trout (A) control and (B) exposed to acid water (pH 4.5) for 8 d. T, thyrotrope.

TABLE 1. Effects of adrenalin and cortisol acetate on plasma cortisol, glucose, and amino acid levels.

Treatment	Killed no. of days after first treatment	Plasma concentration		
		Cortisol ($ng \cdot mL^{-1}$)	Glucose ($mg \cdot dL^{-1}$)	Amino acids ($\mu mol \cdot mL^{-1}$)[a]
None	—	1.1 ± 0.2 (5)	83.9 ± 2.2 (4)	2.97 ± 0.31 (4)
Injected once				
Tween 80	1	79.5 ± 49.7 (3)[b]	138.1 ± 28.5 (3)	—
	2	13.0 ± 5.5 (3)	65.0 ± 11.5 (3)	—
	3	29.1 ± 7.5 (4)[c]	67.2 ± 9.9 (4)	—
	6	19.2 ± 4.1 (4)[d]	72.2 ± 14.3 (4)	—
Adrenalin and cortisol acetate	1	1875.6 ± 392.6 (4)[b]	134.9 ± 31.4(4)	—
	2	1129.8 (2)	122.5 (2)	—
	3	198.5 ± 42.8 (5)[c]	77.9 ± 6.1 (5)	—
	6	51.3 ± 10.2 (5)[d]	81.6 ± 3.8 (5)	—
Injected twice				
Tween 80	6	35.5 ± 7.9 (9)[e,f]	78.2 ± 13.7 (9)	3.26 ± 0.23 (7)
Cortisol acetate	6	1271.9 ± 259.1 (10)[e]	83.0 ± 5.8 (10)	3.06 ± 0.29 (5)
Adrenalin and cortisol acetate	6	1044.3 ± 187.4 (5)[f]	93.7 ± 9.4 (5)	3.75 ± 0.18 (3)

[a]Dashes = not determined.
[b–f]*t*-test, $p < 0.05$–0.001.

Lacroix 1985) including brook trout (Packer and Dunson 1970; Fryer et al. 1988), and decline in plasma osmolarity in low environmental pH was recorded in rainbow trout (Giles et al. 1984). Although the concomitant increase in plasma glucose was usually taken to be part of the mobilization of energy to meet these adverse changes, the source of this extra blood glucose had never been clearly ascertained. In mammals, fat is usually mobilized selectively before protein. In brook trout, this was not likely the case. The simultaneous increase in plasma glucose and amino acid concentrations on day 4 of acid treatment and the accompanying decrease in condition factor suggested that during the first half of the experiment, blood glucose was preferentially derived from tissue proteins and absorbed food at the expense of body growth. The relative weight of the peritoneal fat, which was directly proportional to total body fat content (Tam et al. 1987), decreased significantly after day 30. It is likely that a transition from predominantly protein to fat catabolism occurred. The decline in body weight and condition factor might have provided the stimulus for this transition. Another signal that might have caused the changeover is the elevation in plasma amino acids which induced an increase in liver alanine aminotransferase activity to eliminate the surplus amino acids in order to meet the rigid distribution ratio of proteins and amino acids in the body compartments (Guyton 1986). As a result, plasma amino acid and protein levels declined, and lipid was metabolized. This hypothesis is consistent with the view that fish preferentially generate aerobic energy from protein catabolism (Driedzic and Hochachka 1978). The observed endocrine activities would certainly have enhanced these catabolic changes. The transient rise in blood glucose level observed 3 h after acidification was probably the result of increased catecholamine secretion (Mazeaud and Mazeaud 1981). Subsequently, the high circulating level of cortisol promoted protein catablism into amino acids, which entered the plasma. Reduction in GH and thyroid hormone secretion would suppress the incorporation of amino acids, into protein. Some of these amino acids were responsible for the observed increase in plasma amino acid levels. Some amino acids were probably transformed into glycogen in the liver, which was not observed to be devoid of glycogen in the acid-treated brook trout. The results of experiment 3 strongly suggested that increased plasma levels of adrenalin and cortisol could not account for the three- to sixfold increase in plasma glucose over the control fish. As observed in clinical studies, stress probably inhibited insulin release (Laycock and Wise 1983). Without insulin, the glucose 6-phosphate formed from the transamination and deamination of amino acids would be converted into glucose under the action of glucose-6-phosphatase.

The results of the present investigations demonstrate that in brook trout, acid exposure results in an inhibition of somatic growth and abnormalities in metabolism which may reflect perturbations in the secretion of pituitary hormones. They also confirm that acclimation occurs after a certain period of acid exposure.

Acknowledgements

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Prolactin Cell Cytology, Plasma Electrolytes, and Whole-Body Sodium Efflux in Acid-Stressed Brook Trout (*Salvelinus fontinalis*)

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Twelve-month-old brook trout (*Salvelinus fontinalis*) of both sexes were exposed to acidified water (pH 4.5) for a period of 52 d. Ultrastructural morphometric studies of the prolactin cells of the pituitaries of acid-stressed female trout revealed a reduction, after a latent period of about 1 wk, in prolactin cell area, cytoplasmic area, and nuclear area which gradually recovered to control values by day 52 of acid exposure. In both male and female trout, acid exposure resulted in significant decreases in plasma osmolarity, and plasma sodium, which gradually returned to control values by day 52 of the study. Total plasma calcium was not altered by acid exposure in either male or female trout. For both male and female trout, acid exposure resulted in transient elevations in haematocrit and a prolonged suppression of blood pH. The whole-body sodium efflux for trout introduced into dechlorinated tap water or distilled water was significantly greater at pH 4.5 than at pH 7.35. Possible relationships between the acid-induced inhibition of the secretory activity of the prolactin cells and impairments in osmotic and ionic regulation observed in acid-stressed trout are discussed.

Des ombles de fontaine (*Salvelinus fontinalis*) mâles et femelles, âgés de douze mois, ont été exposés à une eau acide (pH 4,5) pendant 52 d. Des études morphométriques ultrastructurales des cellules à prolactine de l'hypophyse de femelles exposées à un milieu acide ont montré, après une période latente d'environ 1 sem, une réduction de la surface des cellules à prolactine, de la surface du cytoplasme et de la surface du noyau, qui sont revenues progressivement aux valeurs témoins le 52e jour d'exposition. Chez le mâle et la femelle, une exposition à un milieu acide a provoqué des baisses importantes de l'osmolarité plasmatique et du sodium plasmatique, qui sont revenus progressivement aux valeurs témoins le 52e jour de l'étude. Le calcium plasmatique total n'a pas été modifié par une exposition à un milieu acide ni chez le mâle ni chez la femelle. Chez le mâle et la femelle, l'exposition à un milieu acide s'est traduite par des hausses passagères de l'hématocrite et une suppression prolongée du pH sanguin. La perte de sodium corporelle chez l'omble placé dans de l'eau de robinet déchlorée ou de l'eau distillée était significativement plus importante à pH 4,5 qu'à pH 7,35. On analyse les rapports qui peuvent exister entre une inhibition induite par l'acide de l'activité sécrétrice des cellules à prolactine et des modifications de la régulation osmotique et ionique observées chez des ombles exposés à un milieu acide.

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In recent years, considerable attention has been focussed on investigations designed to elucidate the pathophysiological factors responsible for the reduction or disappearance of fish populations in lakes and streams as a result of acid precipitation. In both laboratory investigations (Lee et al. 1983; Giles et al. 1984; Powell and McKeown 1986; Scherer et al. 1986) and field studies (Fromm 1980; Haines 1981; Lacroix 1985) involving a number of fish species, a consistent consequence of low environmental pH has been perturbations in iono(osmo)-regulation. Mortality associated with acute exposures to acid stress is generally considered to result from osmoregulatory failure associated with a loss of electrolytes from the plasma (Leivestad and Muniz 1976).

In teleost fishes, the pituitary hormone prolactin plays a prominent role in iono(osmo)-regulation with actions that promote sodium retention and maintain plasma osmolarity (Bern

TABLE 1. Parameters of water quality.

Parameter	Neutral	Acid-exposed
pH	7.27 ± 0.01	4.55 ± 0.04
Dissolved O_2 ($mg \cdot L^{-1}$)	6.20 ± 0.18	9.41 ± 0.11
Dissolved CO_2 ($mg \cdot L^{-1}$)	10.71 ± 0.48	12.61 ± 0.37
Temperature (°C)	8.6 ± 0.1	9.0 ± 0.1

1983; Hirano 1986). However, the possibility that the decreases in plasma osmolarity observed in acid-stressed fish may reflect impairments in the secretion of prolactin has not been extensively investigated. Recent cytological studies have revealed that acid exposure influences the secretory activity of the prolactin cells and of the pars intermedia PAS-positive (PIPAS) cells of the teleost pituitary, but the influences differ with species. In tilapia (*Oreochromis mossambicus*), acid exposure resulted in a marked increase in the secretory activity of the prolactin cells as assessed by morphometric methods with no noticeable effects on the PIPAS cells (Wendelaar Bonga et al. 1984a, 1984b). In contradistinction, morphometric studies of the pituitary of the goldfish (*Carrasius auratus*) revealed no observable influences of acid exposure for the prolactin cells but a notable increase in the secretory activity of the PIPAS cells (Wendelaar Bonga et al. 1984b). Despite the extensive use of salmonid species in investigations of the effects of low environmental pH on fish physiology, there has been only a single report involving the pituitary gland. In that study, acid exposure resulted in a transient decrease followed by a compensatory increase in secretory activity of the prolactin cells (Notter et al. 1976). Clearly, no consistent pattern relating acid exposure and prolactin secretion has been established for teleost fishes.

In concurrent cytological studies of the pituitary of the brook trout (*Salvelinus fontinalis*), we have been able to determine that acid exposure results in a sustained activation of the corticotropes, but a sustained inhibition of the somatotropes and thyrotropes (Tam et al. 1988). The purpose of the present study was to investigate the ultrastructure and morphometry of the prolactin cells of brook trout, with time following acidification, and to correlate these observations with determinations of plasma osmotic pressure, sodium, calcium, pH, and haematocrit. Due to the expenditures of time required, morphometric analysis of the prolactin cells was restricted to female trout only.

Materials and Methods

Fish Maintenance and Sample Collection

Twelve-month-old sexually immature brook trout (approximately 115 g) were purchased from Goossens Trout Farm (Otterville, Ont.) on January 9, 1986. After a month long acclimation to laboratory conditions, chronic exposure to pH 4.5 (sulphuric acid) began on February 9 (day 0) and the experiment was terminated on April 2 (day 52). The holding facilities used to maintain the trout for this study have been described earlier (Tam and Payson 1986). The water in which the trout were maintained at the University of Western Ontario was City of London, Ontario, tap water. The physical characteristics of the water were as follows: ions ($mg \cdot L^{-1}$): calcium, 59.7; magnesium, 31.4; sodium, 8.98; potassium, 0.95; iron, 0.11; zinc, 0.16; aluminum, 0.04; lead, 0.02; copper, 0.01; cadmium, 0.0001; sulphate, 32.1; nitrate, 0.66; chloride, 0.007; total phosphorus, 0.088; alkalinity as mg $CaCO_3 \cdot L^{-1}$, 82.5; hardness as $CaCO_3 \cdot L^{-1}$, 123.0; conductivity ($\mu mho \cdot cm^{-1}$), 244.0.

Additional parameters (Table 1) for the water were monitored, on almost a daily basis, as a further index of water quality to insure that appropriate levels of oxygen, carbon dioxide, and temperature were maintained. Although slight differences in these parameters were observed for trout maintained in neutral or acidic water, each of these parameters was well within the bounds of accepted values such as not to limit the scope of metabolic activity of the fish. The methods of fish management and acidification of the water to maintain a pH of 4.5 have also been described in detail in another communication (Tam et al. 1987). Fish were sampled 4 d before and on days 1, 5, 8, 15, 24, 32, 38, and 52 following acidification to allow progressive assessments of the chronology of the effects of low environmental pH. To minimize handling stress, the fish were killed by a sharp blow to the head within seconds of capture.

Pituitary Microscopy

For ultrastructural studies of the endocrine cells of the pituitary gland, pituitaries were dissected and prepared for electron microscopy as described in detail elsewhere (Tam et al. 1988).

For morphometric studies of the prolactin cells, electron micrographs were printed at a final magnification of 3240. Total cell area and nuclear area were determined with a digitizing tablet connected to an IBAS I morphometric analyzer. Due to the elongated nature of the prolactin cells, cells were included in the study only when the plane of sectioning was such that the cell contacted the lumen of the follicle, contained a portion of the nucleus, and basal cytoplasm. The cytoplasmic area was determined for each cell by subtracting the nuclear area from the cell area. For each cell type, a minimum of 60 cells was measured from three to five female trout.

Physiological Measurements

Blood was collected from the caudal vessels immediately after sacrifice and blood pH determined with a pH meter equipped with a microprobe combination electrode. To measure haematocrit, blood was collected in a heparinized capillary tube, centrifuged with an IEC haematocrit centrifuge, and determined with an IEC microcapillary haematocrit reader (International Equipment Co., Needham Heights, MA). Plasma was obtained by centrifugation. The plasma was aliquoted and stored at −30°C. Plasma osmolarity was measured with an Osmette osmometer (model 2007; Precision Systems, Framingham, MA). After suitable dilutions with deionized water, plasma electrolyte concentrations were determined by atomic absorption (spectrophotometry) (model AA-5; Varian Techtron Pty., Melbourne, Australia).

To examine the influence of pH and external sodium concentration on whole-body sodium efflux, an acute acid exposure experiment (60 min) was performed. Brook trout that had been maintained in neutral water were placed in small aquaria filled with 4.0 L of either dechlorinated tap water or distilled water of neutral pH or pH 4.5 where they could swim freely. The cover and sides of the aquaria had been previously painted black to minimize disturbance of the fish. The aquaria were maintained at constant temperature by an outer water jacket chilled to 15.0 ± 0.1°C. Water samples (1 mL) were removed from the aquaria at the time of introduction (0 min) and at 15, 30, 45, and 60 min after the trout was placed in the aquarium. Sodium concentrations in the water samples were determined as described in the previous section.

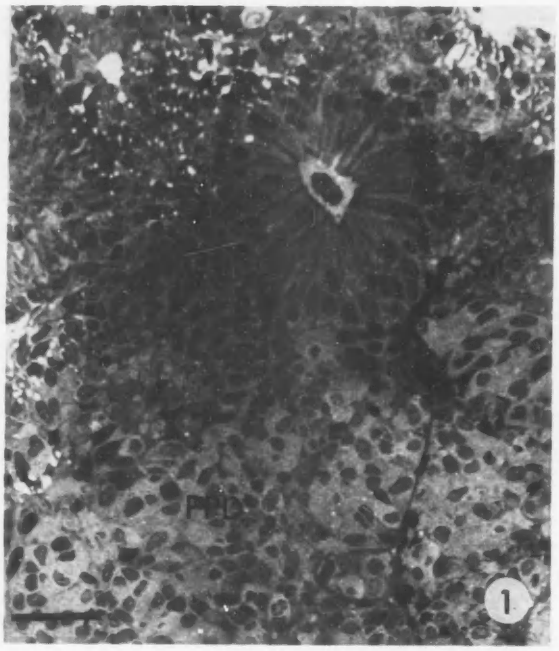

FIG. 1. Light micrograph of a prolactin cell follicle in the pituitary of a control trout. L, follicular lumen: PPD, proximal pars distalis. Scale bar = 50 μm.

Statistical Analyses

For morphometric studies of the prolactin cells, statistical differences between groups were assessed using Student's *t*-test for unpaired observations. When the data were biased, that is not normally distributed, the data were assessed nonparametrically using the Mann–Whitney *U*-test. For comparisons of blood pH, plasma osmolarity, and electrolyte concentrations, Student's *t*-test was used to detect significant differences for pairs of means from acid-treated and neutral fish. In all cases the data were tested for significance at the 5% level and are expressed as mean ± SEM.

Results

During the course of the chronic acid exposure experiment, some parameters of the water chemistry for the trout maintained at neutral pH were assessed as follows: pH, 7.27 ± 0.01 (n = 72); dissolved oxygen, 6.20 ± 0.18 mg·L^{-1} (n = 20); carbon dioxide, 10.71 ± 0.48 mg·L^{-1} (n = 18); temperature, 8.6 ± 0.1°C (n = 91). The same parameters for the acid-exposed trout were as follows: pH, 4.55 ± 0.04 (n = 88); dissolved oxygen, 9.41 ± 0.11 mg·L^{-1} (n = 30); carbon dioxide, 12.61 ± 0.37 mg·L^{-1} (n = 18); temperature, 9.0 ± 0.1°C (n = 91).

Prolactin Cell Cytology and Morphometry

The prolactin cells of the brook trout pituitary form the anterior region of the rostral pars distalis and are arranged in follicles surrounding a central lumen (Fig. 1). In control trout (Fig. 2) the cytological features of the prolactin cells are generally similar to those observed in other salmonids (Cook and van Overbeeke 1972; McKeown and Leatherland 1973; Nagahama et al. 1977). However, a notable feature of the brook trout prolactin cells is the degree of elongation of the cells. Typically the longitudinal diameter of the prolactin cells is 6–8 times the cross-sectional diameter. The luminal surface of the prolactin cells gives rise to numerous microvilli and occasional cilia with the characteristic 9 + 2 arrangement of microtubule doublets. The nucleus is usually located in the basal region of the cell and is separated from the basal plasma membrane by an extensive whorl of rough endoplasmic reticulum. The rough endoplasmic reticulum also appears extensively in lamellae throughout the cytoplasm extending between the nucleus and the lumen of the follicle. The nucleus is ovoid with the long axis parallel to the long axis of the cell and contains a single prominent nucleolus. Numerous spherical secretory granules of uniform electron density can be found throughout the cytoplasm but more frequently around the periphery of the cell. Golgi bodies are abundant and actively form secretory granules. Numerous mitochondria which are elongated in the long axis of the cell are characteristically found in the cytoplasm between the nucleus and the lumen of the follicle.

A striking feature of the brook trout pituitary is the large number of nonsecretory stellate cells which invest the prolactin cells. The stellate cells form a ring around the follicle with their somata situated between the prolactin cells. Stellate cell processes extend between the prolactin cells to the follicular lumen and frequently invaginate the endocrine cells to partition off small areas of cytoplasm in the vicinity of the lumen. Desmosomes and tight junctions are often observed between prolactin cells and stellate cells bordering the lumen of the follicle. Capillaries surround the follicle and its stellate cell investment.

Effects of Acid Exposure

Acid exposure resulted in a reduction in the morphological correlates associated with secretory activity (Fig. 3). This was particularly evident on day 24 following acidification. The prolactin cells were visibly narrower and contained a high density of secretion granules. There was a marked reduction in the amount of rough endoplasmic reticulum in both the apical and basal regions of the cell.

Morphometric analysis of the prolactin cells of trout maintained in water of neutral pH (Fig. 4) revealed no significant change in nuclear area during the course of the experiment. Prolactin cell area increased slightly during the experiment for the trout maintained at neutral pH such that on day 52 of the study, the increase in cell area attained statistical significance ($P < 0.05$) compared with the cell area obtained on day 0.

With acid-exposed trout, morphometric analysis of the prolactin cells (Fig. 4) revealed a progressive reduction, after a latent period of about 1 wk, in cell area and nuclear area. Prolactin cell area and nuclear area reached a minimum on day 24 following acid exposure and then gradually recovered. The recovery in prolactin cell nuclear area was complete on day 52 of the study when nuclear areas were not significantly different from the values obtained for trout maintained at neutral pH on day 0, 24, or 52. The recovery in prolactin cell area was virtually complete by day 52 of the study when cell area was not significantly different from the values obtained for trout maintained at neutral pH on day 0 or 24. However, on day 52 of the study, prolactin cell area of acid-exposed trout was slightly, but significantly, lower than the cell area of the trout maintained at neutral pH.

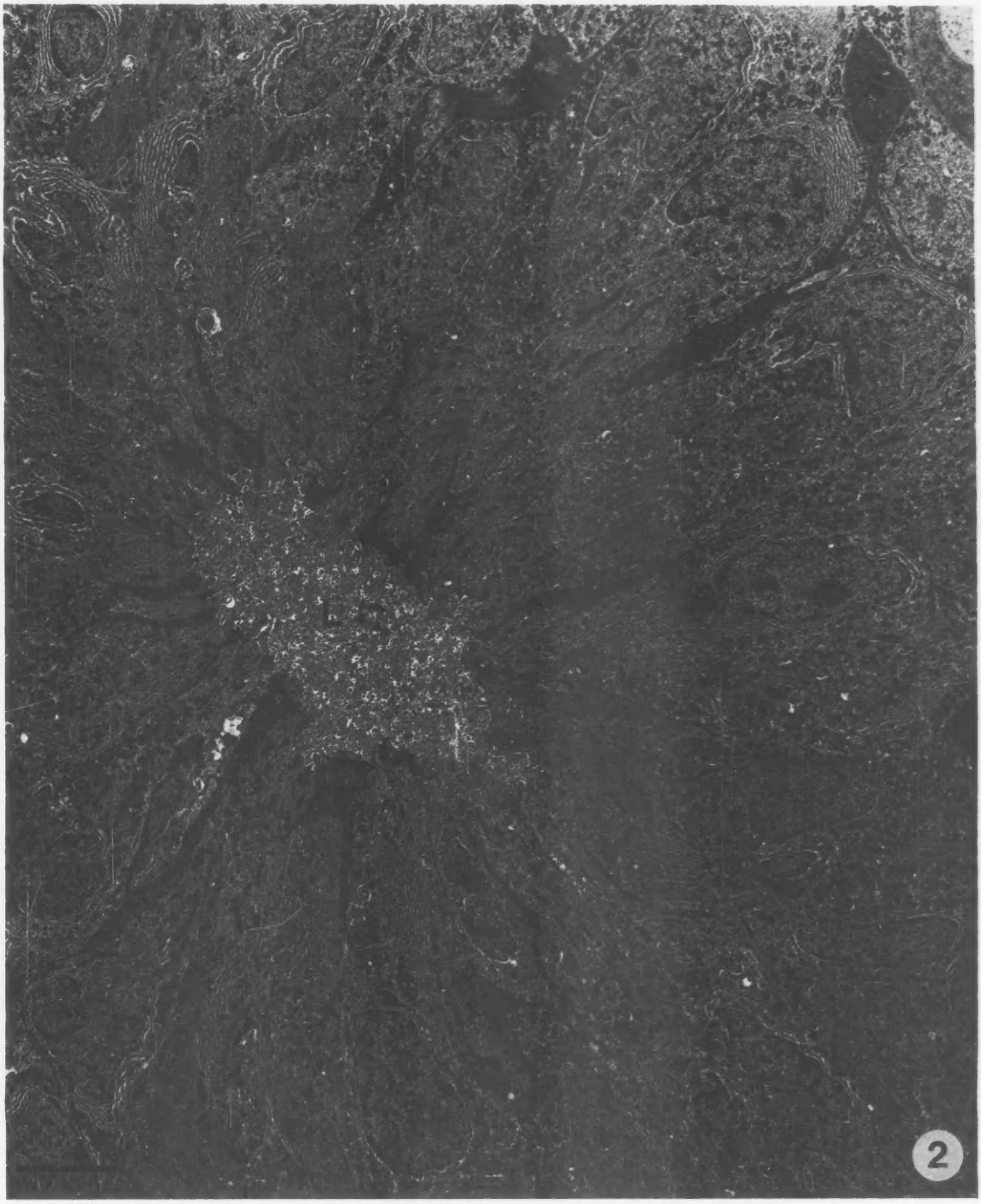

FIG. 2. Prolactin cell follicle of a female control trout. L, follicular lumen; S, stellate cell. Scale bar = 5 μm.

FIG. 3. Prolactin cells of a female trout exposed to acid water (pH 4.5) for 32 d. C, capillary; L, follicular lumen; S, stellate cell. Scale bar = 5 μm.

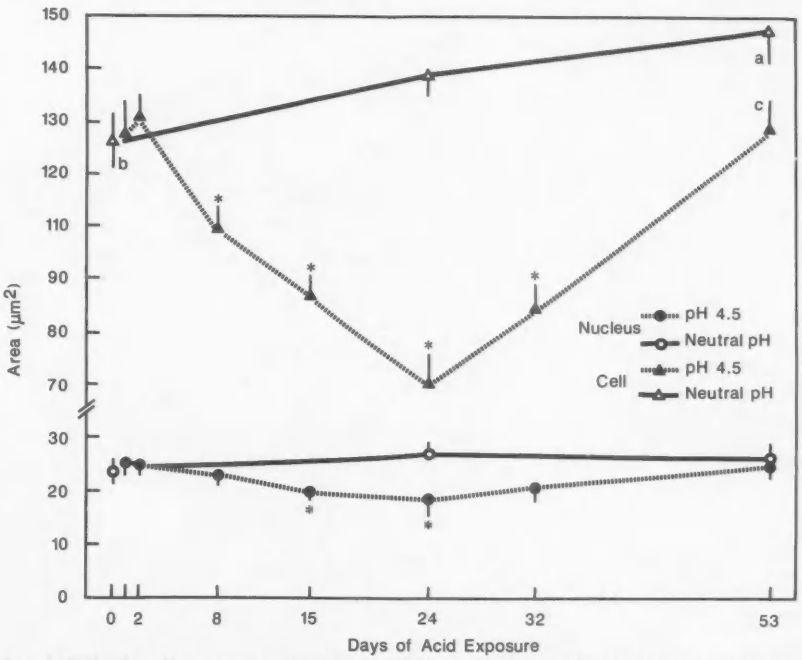

FIG. 4. Prolactin cell areas and nuclear areas, with time, for three to five brook trout maintained in neutral (pH 7.3) or acid (pH 4.5) water. Each point represents the mean ± SEM for a minimum of 60 cells. *significantly lower ($P < 0.05$) than trout maintained at neutral pH; *a*, significantly greater ($P < 0.05$) than *b*; *c*, significantly lower ($P < 0.05$) than *a*.

Blood Chemistry and Haematocrit

Acid exposure resulted in decreases in plasma osmolarity for both male and female trout which became significantly lower on day 8 following acidification (Fig. 5). Plasma osmolarity was restored to control values for female and male trout on days 38 and 52, respectively. Plasma sodium of female trout tended to be reduced after acid exposure with significant decreases on days 8 and 32. For male trout, plasma sodium, after a gradual decline, was significantly reduced from day 8 to day 38 with a return to normal values on day 52 (Fig. 6). Total plasma calcium for both male and female trout was not affected by acid exposure (Fig. 7). Blood pH of both male and female trout exposed to low pH was significantly reduced with a return to normal values in male, but not female, trout on day 52 (Fig. 8). Significant increases in haematocrit for both male and female trout were observed on the fifth day following acid exposure and for male trout were maintained at significantly elevated values on days 8 and 32 (Fig. 9).

Sodium Efflux Experiments

When trout were introduced into dechlorinated tap water of neutral pH (pH 7.35), sodium concentration of the water remained constant for the duration of the experiment (Fig. 10). However, when trout were introduced into dechlorinated tap water in which the pH had been adjusted to pH 4.5, the sodium concentration of the water increased progressively such that at 45 and 60 min following introduction of the fish the increases in sodium were significantly greater than observed with dechlorinated tap water of neutral pH (Fig. 10). When trout were introduced into distilled water of neutral pH (pH 7.35) the sodium concentration of the water increased gradually over the next 60 min. However, when trout were introduced into distilled water in which the pH had been adjusted to 4.5, the sodium concentration of the water increased abruptly and progressively during the 60 min following introduction of the fish. These results demonstrate that whole-body sodium efflux is greater at pH 4.5 than at neutral pH in both dechlorinated tap water and distilled water.

Discussion

A description of the cytological features of the brook trout pituitary has not as yet been reported. In general, the follicular arrangement of the prolactin cells and their ultrastructural features were similar to those observed in other salmonid species (Cook and van Overbeeke 1972; McKeown and Leatherland 1973; Leatherland and Lin 1976; Nagahama et al. 1977). The stellate cells observed in intimate association with the prolactin cells were similar in organization and ultrastructure to those also reported in the salmonid pituitary (Leatherland and Percy 1976; Nagahama et al. 1977). The functional significance of the stellate cells has not as yet been determined. These nonsecretory "glial-like" cells provide a structural framework within the pituitary and display phagocytic activity (Perryman 1983). It has also been suggested that the stellate cells may play a role in the metabolism of pituitary fluids and influence the secretory activity of the endocrine cells (cf. Vila-Porcile and Olivier 1984).

Morphometric determinations of cell and nuclear areas of the prolactin cells of acid-stressed brook trout revealed a reduction, after a latent period of about 1 wk, in the activity of the prolactin

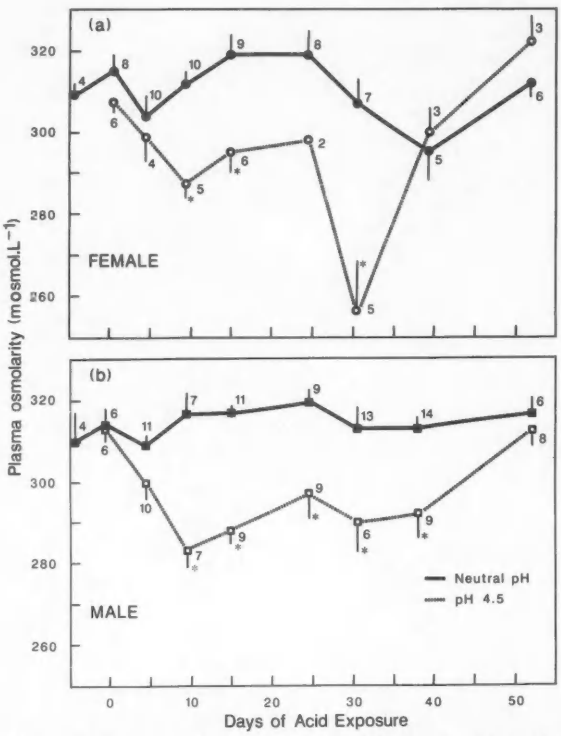

FIG. 5. Plasma osmolarity (mean ± SEM), with time, for (a) female and (b) male brook trout maintained in neutral (pH 7.3) or acid (pH 4.5) water. Numbers adjacent to plotted data denote sample size. *$P < 0.05$.

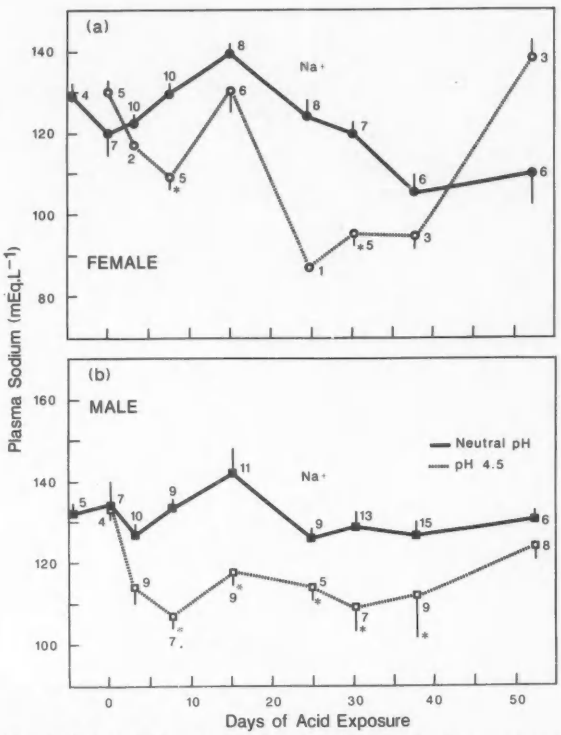

FIG. 6. Plasma sodium (mean ± SEM), with time, for (a) female and (b) brook trout maintained in neutral (pH 7.3) or acid (pH 4.5) water. Numbers adjacent to plotted data denote sample size. *$P < 0.05$.

cells which reached a minimum after 24 d of acid exposure and then gradually returned to control values at the end of the study. In trout as in other teleost fishes the sodium-retaining activity of prolactin is well established (Hirano 1986; Hasegawa et al. 1986), suggesting that prolactin plays an active role in maintaining plasma sodium and osmolarity in salmonid fishes in freshwater. In the present investigations, the activity of the prolactin cells of acid-stressed trout reflected very closely the temporal patterns observed for the reduction and restoration of both plasma sodium and plasma osmolarity. This observation suggests that the reduction in secretory activity of the prolactin cells may be the cause of the hyponatremia and diminished plasma osmolarity observed during acid exposure in brook trout.

The observed changes in haematocrit, blood pH, plasma osmolarity, and plasma sodium for acid-exposed brook trout are consistent with observations of these parameters in salmonids exposed to low environmental pH in both laboratory (McWilliams 1980, 1982; Lee et al. 1983; Giles et al. 1984; Powell and McKeown 1986) and field investigations (Lacroix 1985). Increases in haematocrit of acid-exposed trout have been attributed to a reduction in plasma volume, a mobilization of erythrocytes from the spleen, and erythrocyte swelling (Milligan and Wood 1982). Plasma total calcium was not affected by acid exposure in the present experiments. Exposure of salmonids to low environmental pH has been reported to have little effect on plasma calcium (McKeown et al. 1985) or to reduce plasma calcium (Giles et al. 1984; Lacroix 1985).

An earlier investigation of the effects of acid exposure on prolactin secretion in the brook trout (Notter et al. 1976) revealed that a 2-d exposure to acid water resulted in a transient inhibition of secretion with an atrophy of the prolactin cells and a reduction in prolactin biosynthetic activity as assessed by biochemical indicators of protein synthesis. This inhibition of prolactin cell activity was followed by a compensatory increase in activity on the third and fifth day of acid exposure. In comparing these results with the observations of the present study, the inhibition of prolactin cell activity as a result of acid stress is a common feature. However, the time course of the inhibition of prolactin secretion is quite different and reasons for this difference are not readily apparent. Plasma electrolytes and osmolarities were not reported by Notter et al. (1976) which precludes more direct comparisons with the current work.

Investigations of prolactin cell activity of acid-exposed tilapia and goldfish have yielded differing results. With tilapia (Wendelaar Bonga et al. 1984a), acid exposure resulted in an increase of the secretory activity of the prolactin cells, as assessed by both morphometric and biochemical methods, which attained a maximum at 10 d of acid exposure and was maintained for 30 d after acidification when the experiment was terminated. Acid exposure resulted in decreased plasma osmolarity, sodium, and calcium which returned to control values within a few days. These results suggest that the restoration of plasma electrolytes observed during the course of acid exposure in tilapia was mediated by an enhanced secretion of prolactin. In goldfish (Wendelaar Bonga et al. 1984b), acid exposure had no effect on the secretory activity of the prolactin cells, as

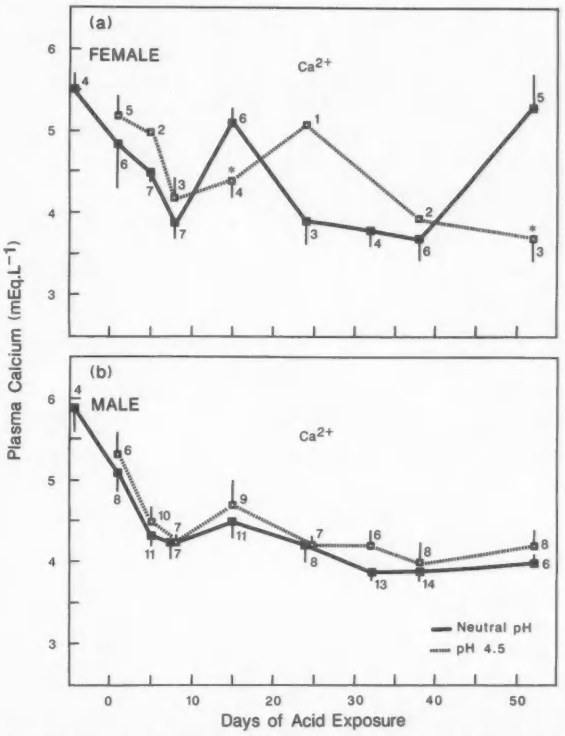

FIG. 7. Plasma calcium (mean ± SEM), with time, for (a) female and (b) male brook trout maintained in neutral (pH 7.3) or acid (pH 4.5) water. Numbers adjacent to plotted data denote sample size. *$P < 0.05$.

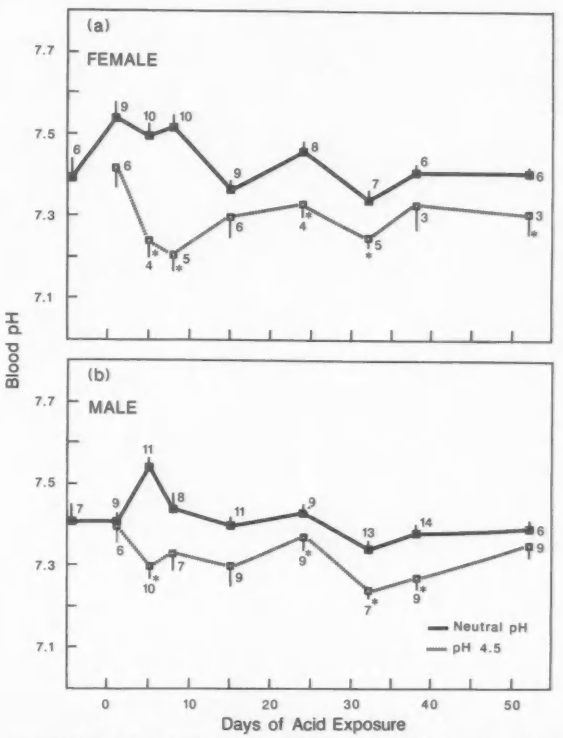

FIG. 8. Blood pH (mean ± SEM), with time, for (a) female and (b) male brook trout maintained in neutral (pH 7.3) or acid (pH 4.5) water. Numbers adjacent to plotted data denote sample size. *$P < 0.05$.

assessed by morphometric methods, by day 14 of acid exposure, despite significant reductions in plasma osmolarity and calcium; the latter showed no indication of restoration towards normal values during the 14-d period of acid exposure.

In the present investigations, acute exposure of brook trout to low pH, in dechlorinated tap water or distilled water, resulted in an increase in whole-body sodium efflux. Exposure to low environmental pH results in a loss of electrolytes from the plasma which probably occurs mainly across the epithelial surfaces of the gills and to a much lesser extent across those of the skin. Acid exposure results in increased branchial permeability to water and ions (McWilliams and Potts 1978; McDonald 1983a, 1983b; Wendelaar Bonga et al. 1984a). The increases in branchial permeability to ions and water may represent a weakening of epithelial intercellular junctions leading to an increase in paracellular ion efflux (Marshall 1985). Morphological studies have shown that prolonged exposure to an acidic environment results in a disruption of the cellular organization of the gill epithelium (Chevalier et al. 1985). The enhanced whole-body sodium efflux of acid-stressed trout demonstrated in the present in vivo studies supports these earlier in vitro investigations. The marked potentiation of whole-body sodium efflux observed for trout in distilled water of low pH in all likelihood reflects the greater transepithelial sodium concentration gradient for gills bathed in distilled water than in dechlorinated tap water.

Although acid exposure resulted in an increase in sodium efflux, there were no significant reductions in plasma osmolarity or plasma sodium in trout sampled on day 1 or 5 after exposure to acid water (Fig. 6 and 7). These findings suggest that the trout were capable of either maintaining or restoring plasma electrolytes, if only on a short-term basis, through some adaptive mechanism. Because acid stress results in an immediate (within minutes) stimulation of the secretion of cortisol and a significant increase in the secretory activity of the corticotropes, observable at least on the first day following acid exposure (Tam et al. 1988), it is possible that the increase in plasma sodium efflux may be offset by osmoregulatory adjustments mediated by adrenocorticosteroid hormones. The increased metabolic costs of maintaining plasma electrolytes during conditions of acid stress may be a significant factor contributing to the reductions in body growth and perturbations in carbohydrate and protein metabolism observed in these trout (Tam et al. 1988).

From the studies of the effects of low environmental pH on brook trout, goldfish, and tilapia, it is evident that the responses of teleost prolactin cells to acid exposure are indeed varied. With particular reference to the present investigations in brook trout, the mechanism by which acid exposure results in an inhibition of prolactin secretion cannot as yet be ascertained. An inhibition of prolactin secretion in response to emotional or physical "stress" is a feature frequently observed in the mammalian pituitary (Neill 1980). The inhibition of prolactin secretion observed in acid-stressed brook trout may very well reflect a similar response to "stress" which presumably overrides any stimulatory influences on prolactin secretion resulting from a

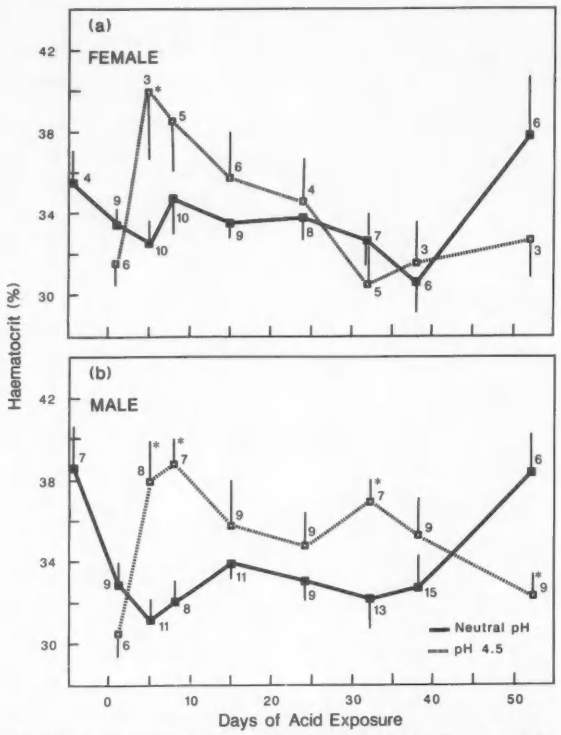

FIG. 9. Haematocrit (mean ± SEM), with time, for (a) female and (b) male brook trout maintained in neutral (pH 7.3) or acid (pH 4.5) water. Numbers adjacent to plotted data denote sample size. $*P < 0.05$.

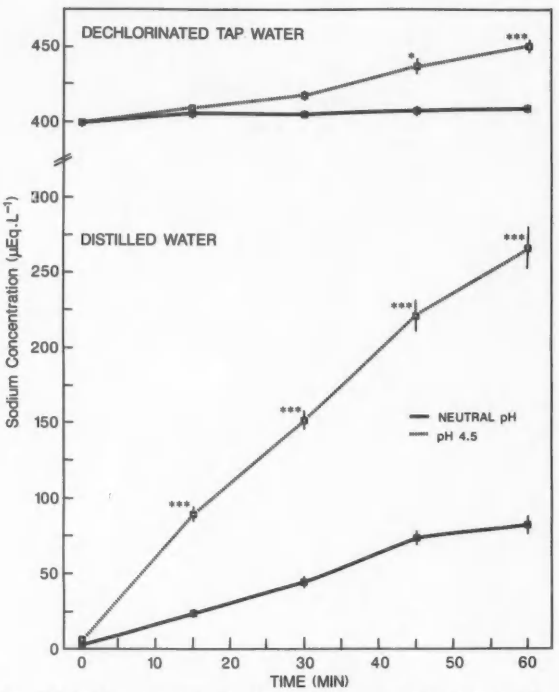

FIG. 10. Sodium concentration (mean ± SEM; $n = 5$), with time, for an aquarium containing 4 L of (a) dechlorinated tap water or (b) distilled water maintained at a neutral pH (pH 7.3) or pH 4.5 following the introduction of individual brook trout. Significantly greater ($*P < 0.05$; $**P < 0.01$; $***P < 0.001$) than trout maintained at neutral pH.

decrease in plasma osmolarity. In view of the prominent role for prolactin in iono(osmo)-regulation in teleost fishes and the disparity in results relating acid exposure to the activity of teleost prolactin cells, more extensive investigations of prolactin secretion in additional species under conditions of acid stress are clearly warranted.

Acknowledgements

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Atmosphere–Ocean Coupling in the Northern Gulf of St. Lawrence: Frequency-Dependent Wind-Forced Variations in Nearshore Sea Temperatures and Currents[1]

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Rose, G. A., and W. C. Leggett. 1988. Atmosphere–ocean coupling in the northern Gulf of St. Lawrence: frequency-dependent wind-forced variations in nearshore sea temperatures and currents. Can. J. Fish. Aquat. Sci. 45: 1222–1233.

We found nearshore sea temperatures and currents on the north shore of the Gulf of St. Lawrence to be linked to wind-forced upwellings and downwellings. Multiple coherence of alongshore and cross-shore wind stresses with sea temperature ($f > 1$/d removed) was significant at periods >3 d (maximum $K^2 = 0.90$) and at 1.8 d. Partial coherences were frequency dependent. At periods >3 d, which contained most of the variance, alongshore forces dominated. Cross-shore winds were significant only at 1.8 d. A time domain model using lagged wind stress and cumulative air temperature as predictors explained 95% of the variance in post-stratification nearshore temperature. Temperature changes were virtually synchronous along 150 km of coastline. Alongshore currents were coherent with alongshore winds at periods >2 d. Cross-shore currents were coherent with cross-shore winds at periods of 1–2 d. These results are compared with Csanady's models of wind-forced coastal thermocline oscillation. We conclude that alongshore winds force major upwellings and downwellings in this region. Cross-shore forces are important at $f > 0.5$/d. These dynamics regulate local primary biological processes and the transport of energy through their influence on fish migration.

Les auteurs ont trouvé que les températures et les courants des eaux côtières de la rive nord du golfe du Saint-Laurent étaient liés à des courants ascendants et descendants provoqués par le vent. La cohérence multiple des contraintes parallèles et perpendiculaires à la rive avec la température de l'eau de mer ($f > 1$/d éliminé) était significative aux périodes supérieures à 3 d (K^2 maximum = 0,90) et à 1,8 d. Les cohérences partielles étaient fonction de la fréquence. Aux périodes supérieures à 3 d, qui présentaient le plus de variance, les forces parallèles à la rive dominaient. Les vents perpendiculaires à la rive n'étaient significatifs qu'à 1,8 d. Un modèle par domaine temporel utilisant comme paramètres de prévision la contrainte éolienne décalée et la température cumulative de l'air a permis d'expliquer 95 % de la variance de la température des eaux côtières après stratification. Les modifications de température étaient pratiquement synchrones le long de 150 km de rive. Les courants et les vents parallèles à la rive étaient cohérents aux périodes supérieures à 2 d. Dans le cas des courants et vents perpendiculaires à la rive, la cohérence était notée aux périodes de 1 à 2 d. Les auteurs ont comparé ces résultats à ceux des modèles de Csanady portant sur l'oscillation provoquée par le vent de la thermocline côtière. Les auteurs concluent que les vents parallèles à la rive sont la cause d'importants courants ascendants et descendants dans cette région. Les forces perpendiculaires à la rive sont importantes à $f > 0,5$ d. Ces facteurs dynamiques régissent localement les processus biologiques primaires et le transport de l'énergie, de par leur influence sur la migration du poisson.

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The importance of coastal upwelling to diverse marine biological processes is well established (e.g. Field et al. 1980; Mysak 1986). Coastal upwelling processes often produce local increases in primary and secondary productivity as well as cooling of coastal waters (Wooster 1981).

The north shore of the Gulf of St. Lawrence exhibits low average levels of primary productivity (Steven 1974) and has been considered to be a biological desert (Dickie and Trites 1983). There is evidence, however, to suggest episodic upwelling along this coastline. Rapid declines in nearshore sea temperature of up to 10°C within a few days occur during the summer (A. Nadeau, Ministère de l'Agriculture, des Pêcheries et de l'Alimentation, Sept-Îles, Qué., pers. comm.). Lauzier et al. (1957) suggested that cold waters observed at times near shore resulted from offshore wind (NW) forced upwelling. Recent examinations of satellite images of sea surface temperature confirmed that nearshore bands of cold water occur periodically during the summer (Lavoie et al. 1986). Lavoie et al. (1986) hypothesized that alongshore wind-driven Ekman transport caused the upwelling of colder deeper waters onshore. The periodic occurrence of patches of high levels of primary productivity, as reported by Steven (1974), is further evidence of upwelling along the north shore.

[1]Contribution to the program of GIROQ (Groupe Interuniversitaire de Recherche Océanographique du Québec).

We have recently undertaken a study whose objective was to account for the spatial and temporal variability in key biological processes on the St. Lawrence north shore. The north shore comprises a vital region for migratory populations of fishes (Jean 1963; Parent and Brunel 1976), sea birds (Chapdelaine and Brousseau 1984), and marine mammals, native not only to the Gulf of St. Lawrence but to adjacent areas of the western Atlantic. This region contains major spawning grounds for capelin (*Mallotus villosus*) (Parent and Brunel 1976) and Atlantic salmon (*Salmo salar*) and is an important feeding area for the northern Gulf Atlantic cod (*Gadus morhua*) stock (Jean 1963; Templeman 1974) and for mackerel (*Scomber scombrus*) (unpubl. data).

A major goal of our research is to account for the variability in the movements and catches of cod. Onshore migrations of cod to the north shore of the Gulf of St. Lawrence have sustained an inshore fishery there for several centuries (Whiteley 1977). The high variability in catch rates, at scales up to years, characteristic of this and other inshore cod fisheries in Atlantic Canada, is thought to result primarily from the variable movements of cod (L. Dumas, President, Lower North Shore Fishermen's Association, Lourdes-de-Blanc-Sablon, Que., pers. comm.; H. Lear, Department of Fisheries and Oceans, St. John's, Nfld., pers. comm.).

Templeman (1966) theorized that cross-shore movements of cod, and the resultant within-year variability in inshore catches along the coast of northeastern Newfoundland, were responses to upwelling induced changes in sea temperature.

We have initiated studies of the dynamics of nearshore currents and temperatures and the forces which regulate them. We believe this to be an essential first step in understanding the variability in many coastal biological processes (Petrie and Drinkwater 1978) including the coastal migrations of fishes (Leggett 1984; Quinn and Leggett 1987).

In this paper we test the hypothesis that wind-forced upwelling and downwelling occurs along the north shore of the Gulf of St. Lawrence. We also describe empirical models that account for the variance observed in nearshore sea temperatures. In an accompanying paper (Rose and Leggett 1988), we use these results to test the theory that cod movements and catches in the nearshore zone are regulated in part by coastal hydrodynamics.

Methods

Ryan thermographs (Ryan Corp., WA) were moored with 50-kg cement blocks at 12 sites along 150 km of shoreline between La Tabatiere and Blanc-Sablon, Quebec, on the lower north shore of the Gulf of St. Lawrence (Fig. 1). All instruments were moored 0.5 m off the bottom at depths ranging from 6 to 25 m. Instruments were calibrated by the Bedford Institute of Oceanography, Dartmouth, N.S. The continuous temperature records were digitized at 4-h intervals and corrected by application of appropriate calibration curves prior to analysis. The temperature records obtained covered the period from June 12 to August 13, 1985.

Aanderaa RCM4 current meters were moored at three sites in the northeastern Gulf for the period July 6 to August 6, 1985 (Fig. 1). Two meters were moored at each site: station A at 16 and 29 m (water depth 54 m), station B at 21 and 36 m (water depth 41 m), and station C at 19 and 30 m (water depth 35 m). These mooring depths were instrument determined and are accurate to 1 m. Recorded instrument depths varied by no more than ± 0.5 m during the record. Vector components of current were resolved in relation to alongshore ($+v = 60°$ true) and

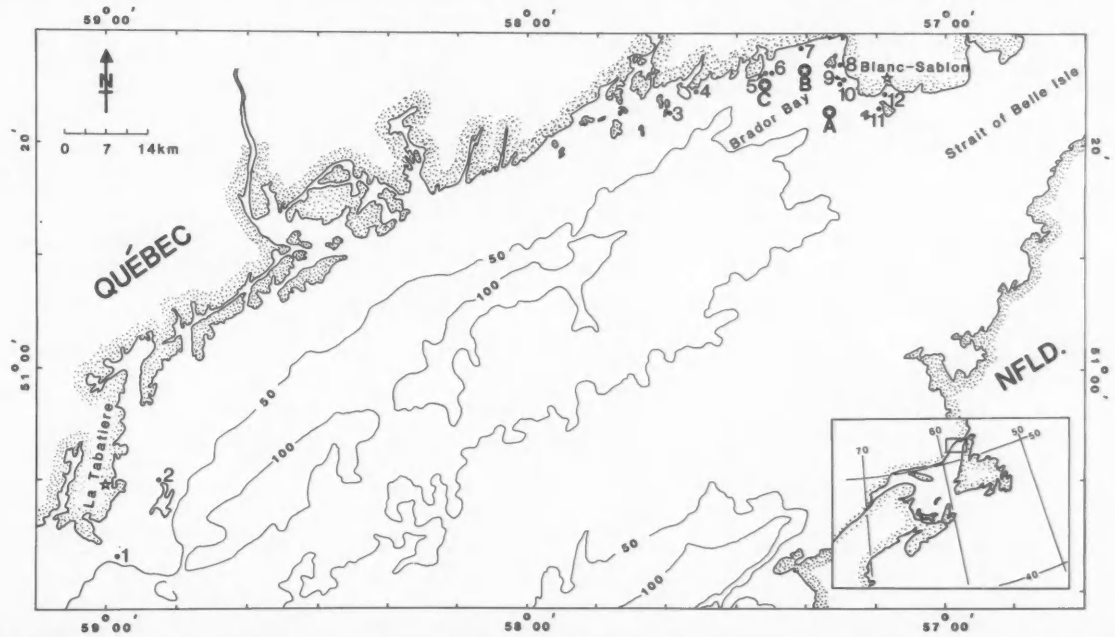

FIG. 1. North shore of the Gulf of St. Lawrence from La Tabatiere to the Strait of Belle Isle. Chevery meteorological station is located on the north shore approximately 50 km outside the study area, to the west of La Tabatiere. Ryan thermograph mooring sites indicated with numbers. Current meter moorings indicated with stars/letters. Isobaths in fathoms (1 fathom = 1.8 m).

cross-shore ($+u$ = 150° true) components. These directions are averages for the study area.

An average of three expendable bathythermograph (XBT Sippican Corp., Massachusetts) temperature profiles were taken daily from June 15 to August 8 in waters with depths ranging from 15 to 120 m in and adjacent to Brador Bay in the northeastern Gulf.

Hourly wind and air temperature data for stations at Blanc-Sablon (in the immediate study area) and Chevery (on the north shore approximately 50 km to the southwest of the study area) were obtained from the Atmospheric Environment Service, Downsview, Ont. Wind vectors were decomposed into longshore ($+v$ = 60° true) and cross-shore ($+u$ = 150° true) components. To assess patterns in wind-forced hydrographic variability, hourly wind values from the two meteorological stations were vector averaged. Wind values to be compared with sea temperature were averaged over 4-h intervals to match the sea temperature sampling frequency.

Units of measurement (SI) used in this paper are as follows: temperature (degrees Celsius); wind speed (metres per second) and stress identified by the direction of origin; current (metres per second) identified by the direction of travel. Wind speeds were converted to wind stress:

$$t_y = 0.000002W \cdot W_y$$

where t_y = wind stress along axis y (alongshore or cross-shore) (square metres per square second) W = wind speed (<7) (metres per second), and W_y = component of wind vector along axis y (metres per second) (Csanady 1982).

All time series data (sea temperature, current vectors, wind vectors) were lowpass filtered to remove fluctuations having periods <24 h (Godin 1972). The high-frequency signals removed included diurnal and semidiurnal tidal components.

The statistical analyses performed consist primarily of spectral analyses (Jenkins and Watts 1968; Koopmans 1974). We use coherence to assess correlation at frequencies <1/d. Coherence (K) takes values between 0 and 1 and is the frequency domain analogue of the simple correlation coefficient. We use coherence squared as the analogue of the coefficient of determination to assess the amount of variance explained. All frequency and time domain analyses were carried out on programs written in regression analysis of time series (RATS) language (VAR Econometrics Inc., Evanston, IL). Multivariate frequency response models, which are the frequency domain analogue of multiple and partial regression in the time domain, were carried out on programs written in RATS according to theory and applications given in Jenkins and Watts (1968) and Koopmans (1974).

Prior to Fourier transformation and the computation of raw periodograms, two types of data transformation were used (Jenkins and Watts 1968). First, nonstationary series (mean and variance not constant over time) were subject to single period difference transformation. Second, the initial and final 25% of each series was cosine tapered to reduce frequency leakage (Jenkins and Watts 1968). In computing spectra from the raw periodograms, tent-shaped smoothing windows were used where

$$S(j) = \sum_{-(n-1)}^{n-1} w_k I(j+k)$$

where $S(j)$ = smoothed series, $n = (m + 1)/2$ where m = window width, and $w_k = n - |k|$ where $-(n-1) < k < (n-1)$ and $\Sigma k = 1$. Window widths were chosen to yield bandwidths equivalent to periods of approximately 24 h, the narrowest detail of interest in the spectra (Jenkins and Watts 1968). Significance and confidence intervals of coherences and phases were assessed using tables given in Koopmans (1974) and Jenkins and Watts (1968). Statistical significances were assessed at the P = 0.05 level unless indicated otherwise.

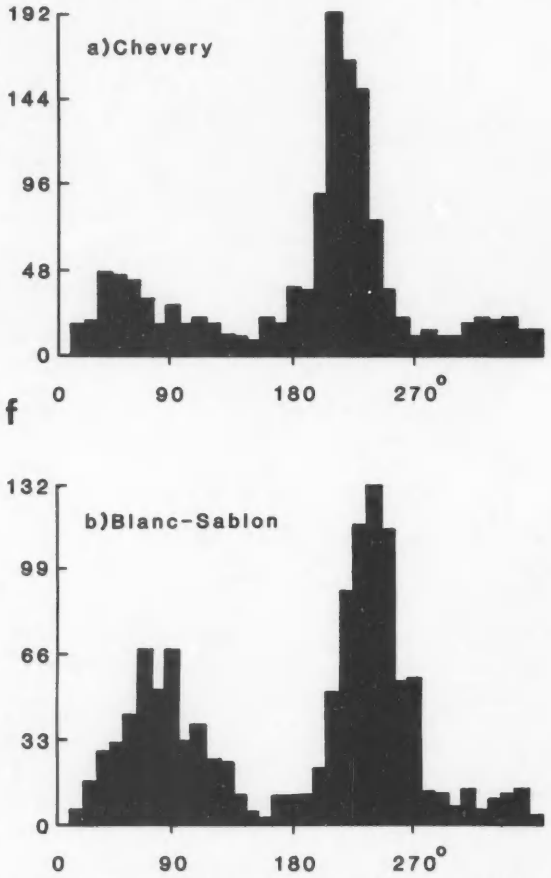

FIG. 2. Occurrence of wind directions (hours) at Chevery and Blanc-Sablon meteorological stations during the study period.

Results

Wind

Wind directions at the Blanc-Sablon and Chevery meteorological stations during the study period were similar and bimodal in distribution (Fig. 2). Major sectors were NE (40–90°) and SW (210–270°). The coastline of the study area lies on average at 60–240°. Thus, SW winds contained strong longshore and offshore components and NE winds contained strong longshore and onshore components. The frequency of NE winds at Blanc-Sablon was somewhat greater than at Chevery.

Alongshore and cross-shore winds were highly coherent (K = 0.82–0.99) at all periods >24 h (Fig. 3). Alongshore led cross-shore winds at periods between 4 and 21 d by an average

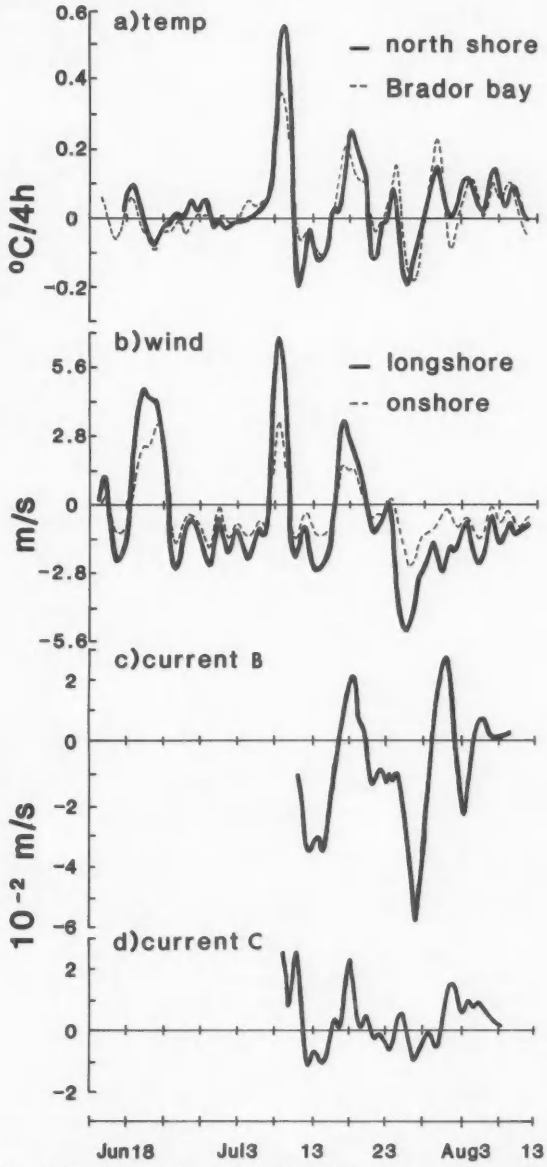

FIG. 3. For the north shore of the Gulf of St. Lawrence during 1985: (a) mean change in sea temperature per 4 h at depths ranging from 6 to 25 m along 150 km of the north shore (n = 12) and at Brador Bay (n = 5); (b) vector-averaged alongshore (NE = +) and cross-shore (onshore = +) components of wind at Chevery and Blanc-Sablon; (c) depth-averaged alongshore current (SW = +) at stations B and C at Brador Bay. For site locations see Fig. 1.

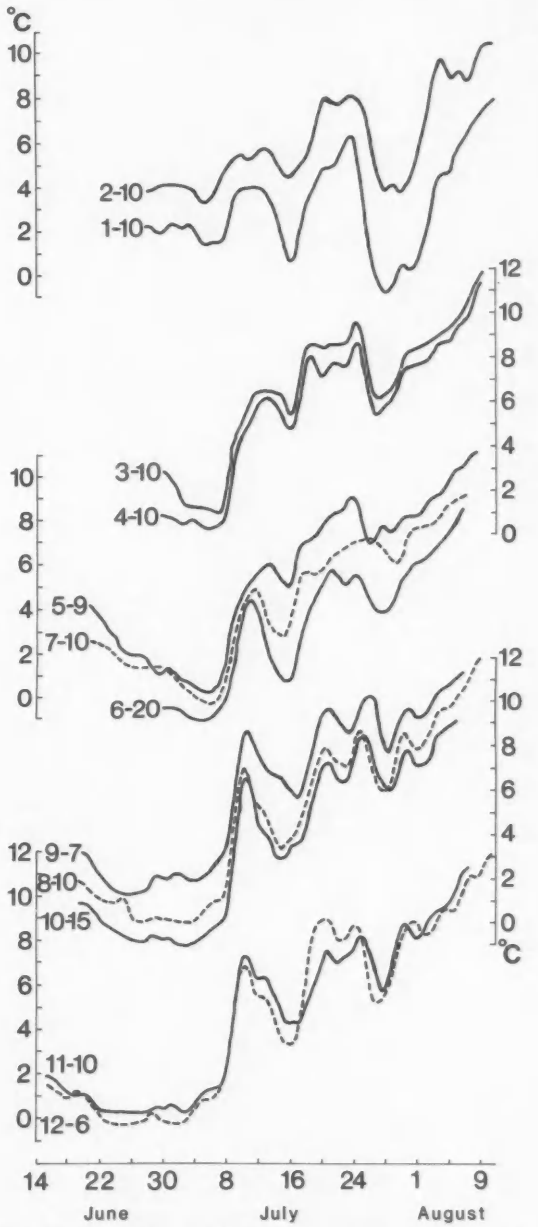

FIG. 4. Thermograph records from 12 sites on the north shore of the Gulf of St. Lawrence during 1985 (for locations see Fig. 1). 1–10 indicates site 1, depth 10 m. Groupings based on cluster analysis of regression coefficients.

of 55° (15–77 h). At periods between 2 and 4 d, alongshore winds led by approximately 30° (4–8 h). At lesser periods, phase leads were zero.

Sea Temperatures

Stratification of the water column in the northeastern part of the study area was first evident at the end of June. Prior to that time, temperatures at all depths were near zero. Waters warmed earlier at the western end of the study area. There, nearshore temperatures were several degrees above zero prior to July 1. No temperature profiles were available to confirm the onset of stratification in this area.

Overall patterns of temperature in the nearshore zone were similar at all sites (Fig. 4). All sites exhibited an overall warming trend beginning during the first week of July and two major

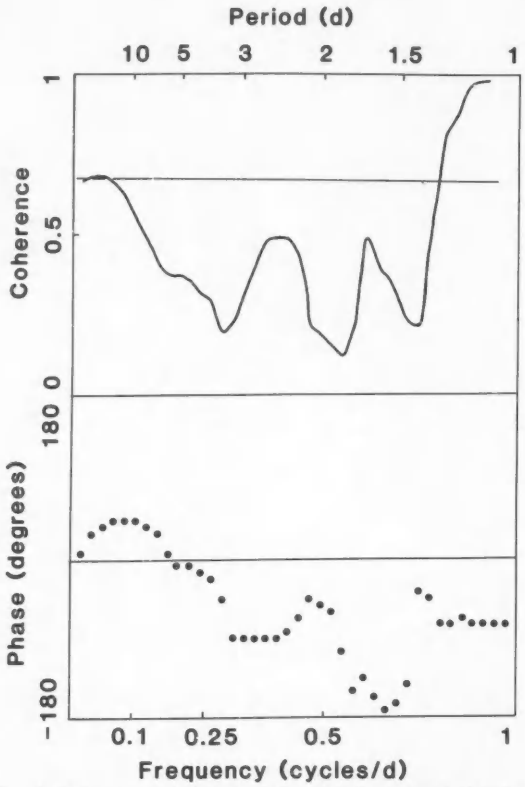

FIG. 5. Coherence and phase leads between thermograph sites 1 and 12 on the north shore of the Gulf of St. Lawrence during 1985. Line across coherence plot indicates coherence > 0 ($P < 0.05$). Confidence limits for the phase spectrum (95%) are approximately $\pm 19°$ at periods >10 d (Jenkins and Watts 1968).

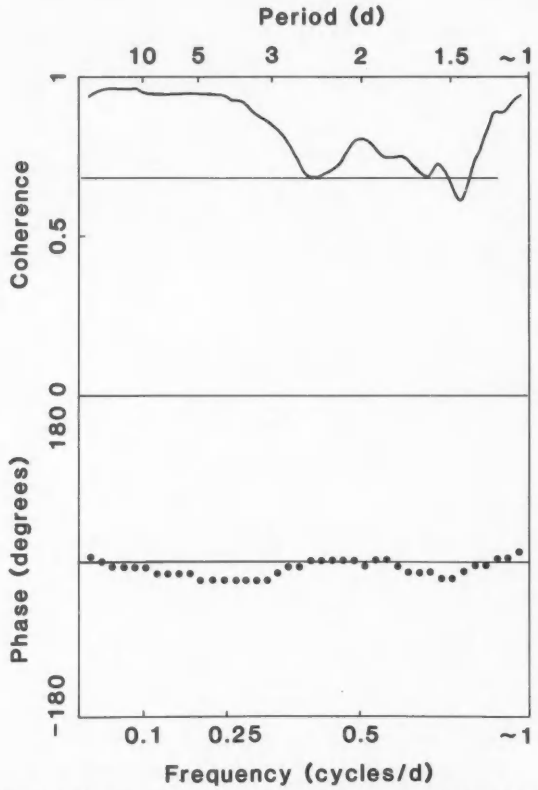

FIG. 6. Coherence and phase leads between thermograph sites 10 (15 m) and 9 (7 m) at adjacent sites on the north shore of the Gulf of St. Lawrence during 1985. Line across coherence plot indicates coherence > 0 ($P < 0.05$). Confidence limits for the phase spectrum (95%) are approximately $\pm 3°$ at periods >3 d (Jenkins and Watts 1968).

cooling events during the second and last weeks of July. Power spectra of sea temperatures indicated that variance peaked at periods of approximately 10 d but was substantial over the range of periods >2 d (e.g. see Fig. 9). All pairs of temperature series exhibited peaks in coherence at periods between 9–15, 4–5, and 2–3 d.

Thermograph sites fell into five groups based on a cluster analysis of the correlation coefficients of their temperature series (Fig. 4). Coherence was frequency dependent and related to the distance between sites. For example, only low-frequency variations (periods >10 d) were coherent at sites 1 and 12, located 150 km apart (Fig. 5). Phase leads indicated that site 1 led site 12 by 24–32 h at these frequencies. At adjacent sites, both high- and low-frequency variations were coherent (Fig. 6).

Sea temperatures were also related to depth. For example, temperatures at two depths at adjacent sites were in each case coherent across a wide range of frequencies. The deeper sites exhibited small (nonsignificant) phase leads over the shallower sites (Fig. 6).

Temperature profiles indicated that rapid temperature changes occurred to depths of 30–40 m (Fig. 7). Temperature records from the deeper current meters also revealed this response (Fig. 8). Thermocline depth changed constantly. During periods of rapid declines in nearshore sea temperatures, temperatures approached those found in deeper waters offshore; during periods of rapid increases, nearshore sea temperatures approached those of surface temperatures offshore.

Wind and Sea Temperatures

Nearshore sea temperatures were coherent with alongshore and cross-shore wind stresses across a wide range of periods >2 d and at approximately 1.8 d (Fig. 9). Sea temperatures were more coherent with wind itself at many of these frequencies. However, wind was far less coherent with currents than was wind stress, so we report analyses based on wind stress here. All subsequent references to wind are to wind stress unless stated otherwise.

Partial coherence analysis was used to assess the independent effects of the highly coherent alongshore and cross-shore wind stresses on sea temperatures. Alongshore winds had higher partial coherence (independent of cross-shore effects) than did cross-shore winds (independent of alongshore effects), at all periods above 2 d (Fig. 9). At periods of less than approximately 1.5 d, by contrast, partial cross-shore effects were equal to those in the alongshore direction. Multiple coherence analysis indicated that over 70% of the variance in nearshore sea temperatures at periods between 2 and 16 d and at approximately 1.5 d could be explained by the joint effects of alongshore and cross-shore winds (Fig. 9).

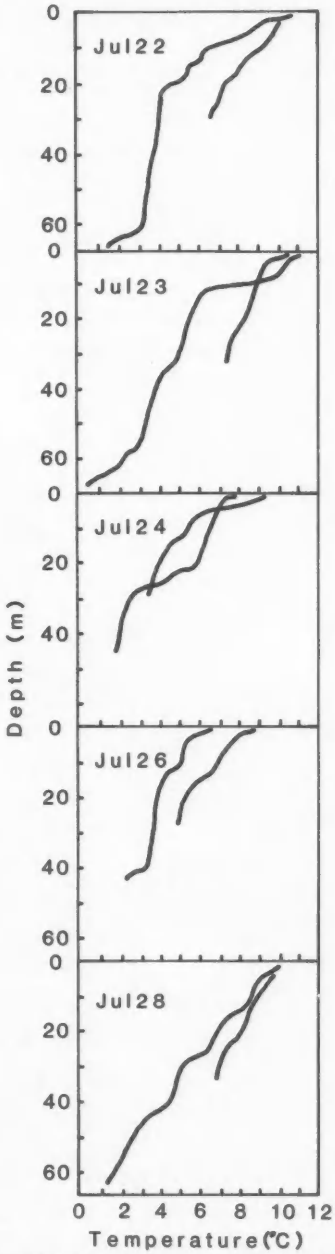

FIG. 7. Expendable bathythermograph profiles before, during, and after a major sea temperature decline (July 22–28) at Brador Bay. The 28-m profiles represent a single site; the deeper profiles represent several sites within a circular zone with radius 1.5 km.

Phase leads indicated that alongshore winds led sea temperature changes by approximately 90° at the longer periods (24–90 h over the range of periods between 3 and 15 d) (Fig. 9). Phase leads increased with decreasing periods so that at periods of <2 d, winds and temperature were virtually 180° out of phase. Cross-shore winds also led sea temperature changes, but by slightly less than did alongshore wind stress at periods >2

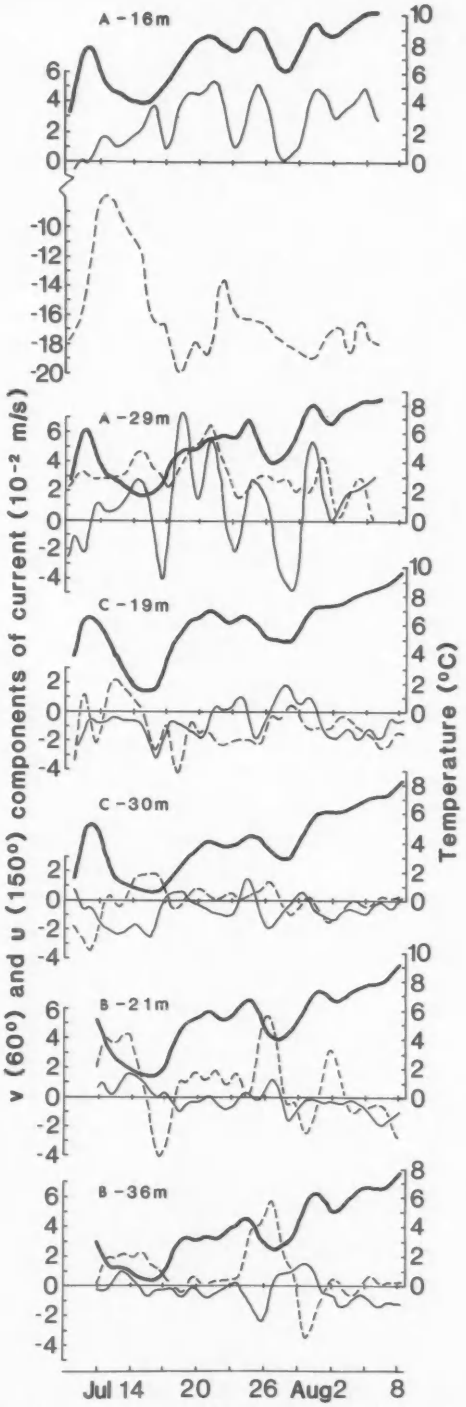

FIG. 8. Alongshore (broken line) and cross-shore (solid line) components of current, and temperature (heavy line), from current meter stations off Brador Bay and near the entrance to the Strait of Belle Isle during 1985 (for locations see Fig. 1). All data lowpass filtered. A-16 m indicates station A, depth of mooring 16 m.

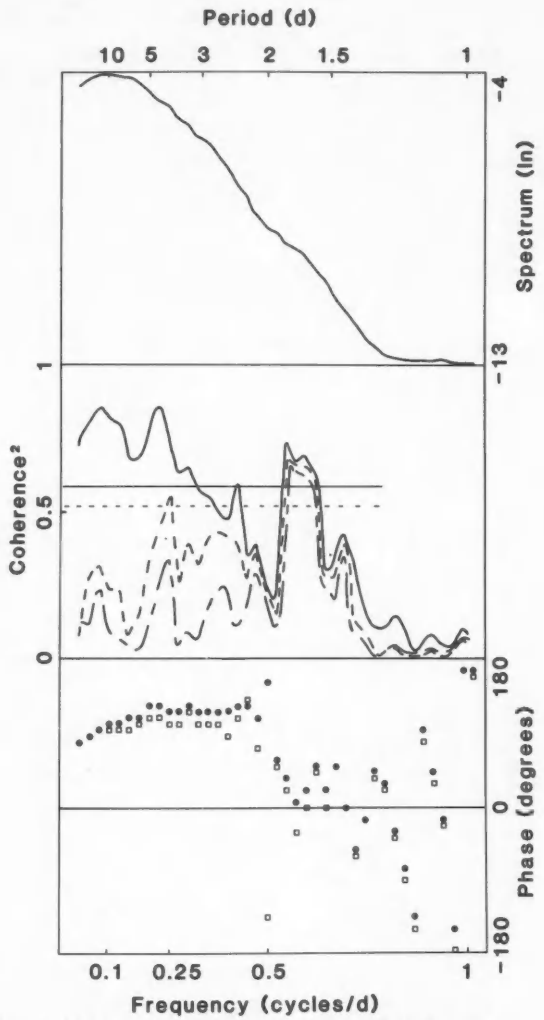

FIG. 9. Coherences and phase leads between alongshore and cross-shore wind stresses and mean nearshore sea temperature (n = 12) along the north shore of the Gulf of St. Lawrence during 1985. Spectrum indicates distribution of variance over frequency for nearshore sea temperature. Coherences squared are multiple between both wind stresses and temperature (solid line) and partials between alongshore stress and temperature (broken line) and cross-shore stress and temperature (uneven broken line). Solid line across coherence plot indicates multiple coherence > 0; broken line indicates partial coherence > 0 ($P < 0.05$). Confidence limits (95%) for phase spectra (solid = alongshore, open = cross-shore) are approximately ±17° for alongshore phase and ±23° for cross-shore phase at periods >2 d (Jenkins and Watts 1968).

d. At the coherence peak at periods of 1.8 d, wind stresses in both directions were not significantly out of phase with temperature, indicating that SW and offshore winds generated immediate sea temperature declines.

We used multiple regression to develop a time domain model of post-stratification mean nearshore sea temperature, calculated each 4 h, over the 150 km of coastline sampled (Table 1). In accord with the results of the spectral analyses, we entered alongshore wind stress at lead times of 24–96 h and cross-shore wind stress at lead times of 24–48 h, in addition to cumulative post-stratification mean air temperature and a constant, into a stepwise selection model (Table 1). Three leads of alongshore wind stress (52, 72, and 96 h) and a single lead of cross-shore wind stress (24 h) were selected as predictors of sea temperature. A multiple regression model using these predictors explained over 95% of the observed variation in post-stratification nearshore sea temperatures (Fig. 10). Residuals from this model ranged from 1.2 to −1.0°C.

TABLE 1. Coefficients of a multiple regression model describing post-stratification nearshore sea temperature on the north shore of the Gulf of St. Lawrence (mean of 12 thermographs). Predictors are cumulative mean air temperature beginning when water stratifies (Cat), alongshore wind stress (Along), and cross-shore wind stress (Cross) at lags selected by stepwise technique. For this model, R^2 = 0.95, frequency of observation = 4/h, n = 228, df = 222. Lags indicate temperature follows. All variables are significant ($P < 0.05$).

Variable	Lag (h)	Coeff.	SE	t
Constant	0	1.1383	0.0868	13.1
Cat	24	0.0034	0.0001	65.5
Along	52	2.4342	0.2822	8.6
Along	72	1.7697	0.2871	6.2
Along	96	2.2812	0.2393	9.5
Cross	24	11.2892	1.0113	11.2

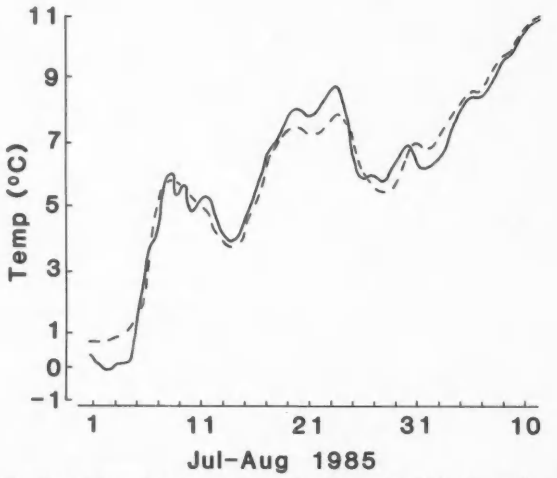

FIG. 10. Observed (broken line) and predicted (solid line) nearshore sea temperatures along the north shore of the Gulf of St. Lawrence during 1985. Predicted values are output from the multiple regression given in Table 1.

Currents

At station B, located near the eastern border of Brador Bay (Fig. 1), alongshore currents dominated at both 21 and 36 m, but current directions fluctuated widely between SW and NE (Fig. 8). Alongshore currents at the two depths were coherent at periods >3 d (K = 0.62–0.71) and at 1.7 d (K = 0.60) and out of phase by 20–25° (2–22 h over the range of periods 1.7–15 d, shallow leading at all periods). Cross-shore currents at the two depths were incoherent except at 2.5 d (K = 0.75, in phase).

At station C, located on the western margin of Brador Bay, current directions were similar to those at station B, but the

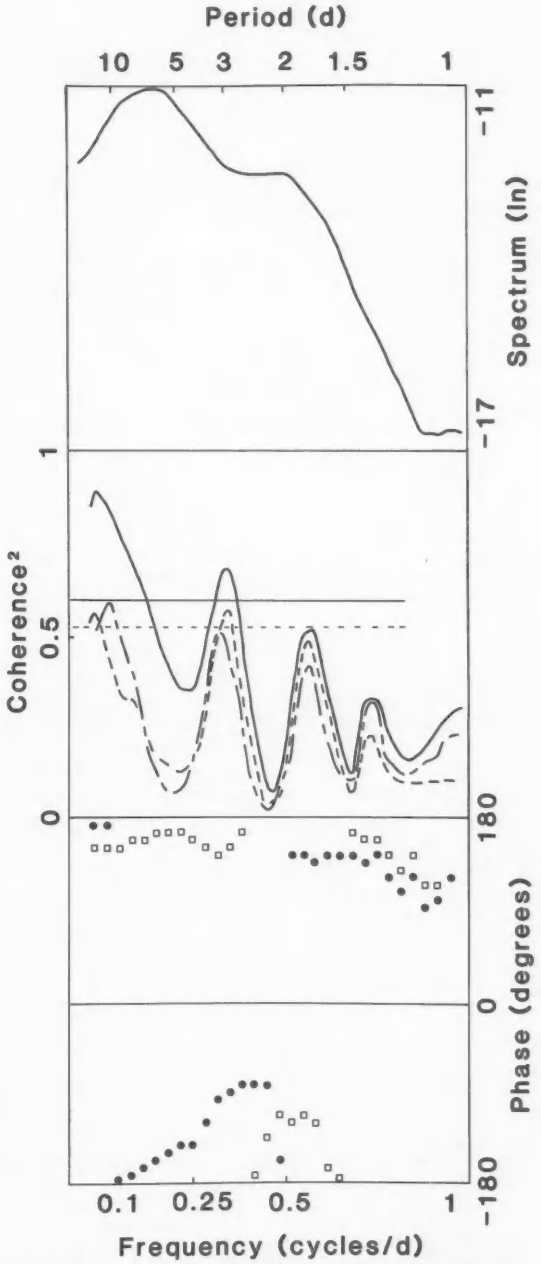

FIG. 11. Coherences and phase leads between alongshore and cross-shore wind stresses and vector-averaged alongshore currents at stations B and C at Brador Bay during 1985. Spectrum indicates distribution of variance over frequency for alongshore currents. Coherences squared are multiple between both wind stresses and current (solid line) and partials between alongshore stress and current (broken line) and cross-shore stress and current (uneven broken line). Solid line across coherence plot indicates multiple coherence > 0; broken line indicates partial coherence > 0 ($P < 0.05$). Confidence limits (95%) for phase spectra (solid = alongshore, open = cross-shore) are approximately ±5° at periods of 10 d, ±23° at periods of 5 d, and ±19° at periods of 3 d (Jenkins and Watts 1968).

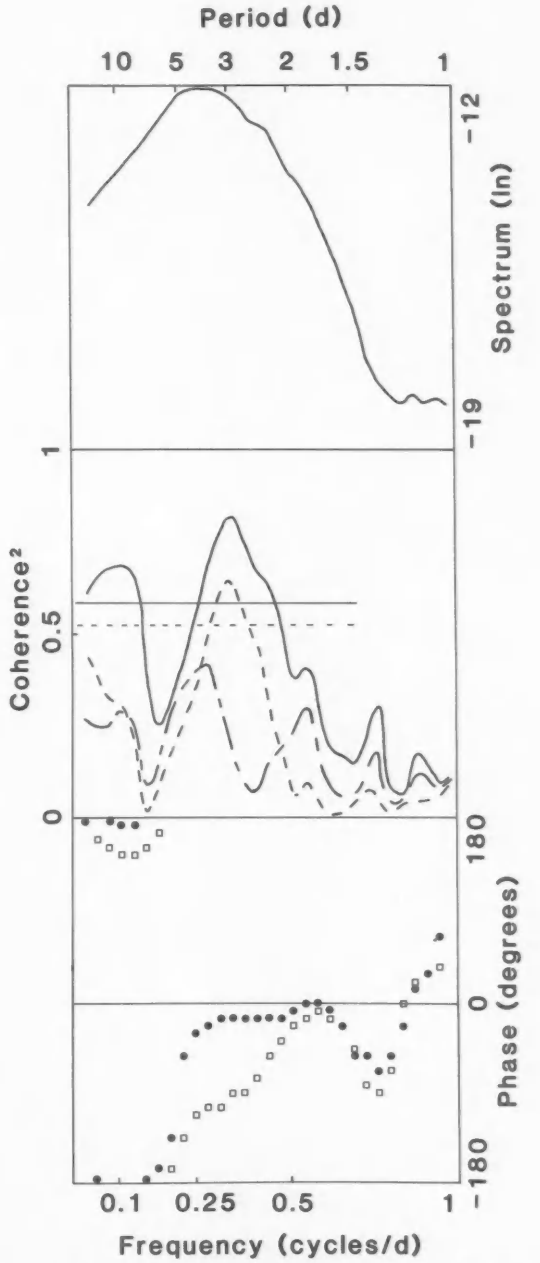

FIG. 12. Coherences and phase leads between alongshore and cross-shore wind stresses and cross-shore currents at 21 m of depth at station B at Brador Bay during 1985. Spectrum indicates distribution of variance over frequency for currents. Coherences squared are multiple between both wind stresses and current (solid line) and partials between alongshore stress and current (broken line) and cross-shore stress and current (uneven broken line). Solid line across coherence plot indicates multiple coherence > 0; broken line indicates partial coherence > 0 ($P < 0.05$). Confidence limits (95%) for phase spectra (solid = alongshore, open = cross-shore) are approximately ±20° at periods >7 d and ±15° at periods near 3 d (Jenkins and Watts 1968).

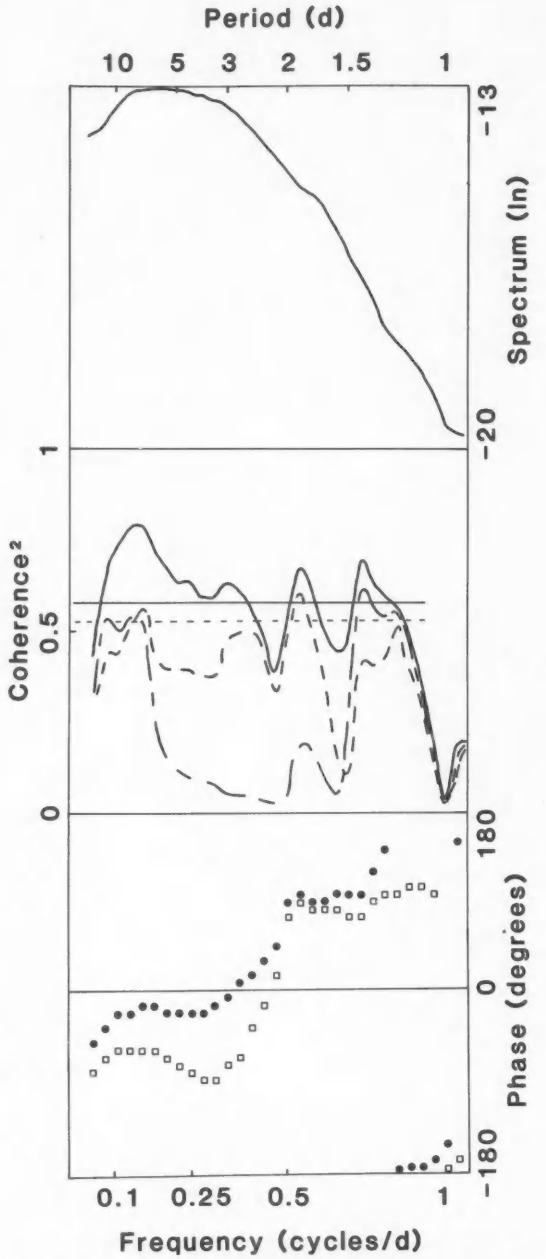

FIG. 13. Coherences and phase leads between alongshore and cross-shore wind stresses and cross-shore currents at 36 m of depth at station B at Brador Bay during 1985. Spectrum indicates distribution of variance over frequency for currents. Coherences squared are multiple between both wind stresses and current (solid line) and partials between alongshore stress and current (broken line) and cross-shore stress and current (uneven broken line). Solid line across coherence plot indicates multiple coherence > 0; broken line indicates partial coherence > 0 ($P < 0.05$). Confidence limits (95%) for phase spectra (solid = alongshore, open = cross-shore) are approximately ±22° for alongshore stress at periods >3 d and 1.8 d and for cross-shore stress at periods of 1.5 d (Jenkins and Watts 1968).

amplitude of current oscillations was lower (Fig. 8). The alongshore currents at the shallow and deep meters were coherent at periods of 3 d ($K = 0.60$). Phase leads indicated that bottom currents led surface currents by approximately 55° (11 h).

At station A (water depth 50 m), located closest to the Strait of Belle Isle and beyond the outer boundary of Brador Bay, alongshore currents were incoherent with those at stations B and C. Here, the currents recorded at 16 m were dominated by a SW alongshore component throughout the study period that averaged between 0.1 and 0.2 m/s (Fig. 8). The alongshore currents recorded at 29 m were NE and averaged 0–0.06 m/s. Cross-shore currents at this station had peak coherence with alongshore (not cross-shore) currents at station B at periods of 2.5 d ($K = 0.50$, $P < 0.10$). Cross-shore currents at the 16 and 29 m depths were coherent at periods between 2 and 14 d (minimum $K = 0.65$). Peaks in coherence occurred at periods of 2.6 and 4.7 d ($K = 0.78$ and 0.81, respectively). Phase leads indicated that at the coherence peaks of 2.6 and 4.7 d the deeper currents led shallower currents by 35° (6 h) and 13° (4 h), respectively.

Wind and Currents

We report the analyses of the station B data as being representative of nearshore current conditions. Coherences and phase leads of wind stress and currents at stations B and C were similar.

Multiple coherence of alongshore and cross-shore wind stresses and nearshore alongshore currents was significant at periods >6 d and at approximately 3 d (Fig. 11). Higher frequencies were not coherent. The power spectrum indicated that variability in these currents peaked at periods between 5 and 10 d (Fig. 11). Partial coherences of alongshore and cross-shore wind stresses with alongshore currents were nearly identical (Fig. 11). Winds and currents were virtually 180° out of phase at all periods, indicating that current response to winds was almost immediate (Fig. 11).

Wind stresses were also coherent with nearshore cross-shore currents. At the near surface meters, multiple coherence was significant at periods >7 d and between 2 and 4 d (Fig. 12). Variance was maximum at periods between 2 and 7 d (Fig. 12). Partial coherences indicated that alongshore wind stress was significantly coherent with currents at the periods of maximum variance, independent of cross-shore winds (Fig. 12). Partial coherences of cross-shore winds and currents were not significant at any period but exceeded those of alongshore winds at periods of <2 d.

At the deep meters, multiple coherences of alongshore and cross-shore wind stresses and cross-shore currents were significant over a wide range of periods (Fig. 13). Variance was widely distributed but maximum at periods >2 d (Fig. 13). Partial coherences between alongshore wind stress and currents were much greater than between cross-shore winds and currents at the longer periods, but cross-shore partial coherences were greater at periods of <1.5 d (Fig. 13).

Phase leads differed between the surface and deeper currents. At the longer periods (>4 d), alongshore wind stress was virtually 180° out of phase with surface cross-shore currents (Fig. 12). This indicates that SW winds were followed closely by the seaward movement, and NE winds by shoreward movement, of surface waters. Deeper currents, by contrast, were in phase with alongshore winds at these periods, indicating that SW winds were followed closely by shoreward movements, and

NE winds by seaward movements, of deeper waters (Fig. 13). At the shorter periods between 2 and 4 d, alongshore winds and cross-shore currents were in phase near the surface but winds led bottom currents by 90° (12–24 h). At the shortest periods (1.5–2 d), where coherences between cross-shore winds and currents exceeded those between alongshore winds and cross-shore currents, cross-shore winds led currents by approximately 90° (10 h at 1.8 d).

At station A, at the entrance to the Strait of Belle Isle, wind stresses and currents were not significantly coherent. The persistant SW surface flows here did appear to have some association with alongshore wind stress (maximum $K = 0.60$ at 5 d, $P > 0.10$, 180° out of phase). The strong SW winds which began on July 10 coincided with a major recession of the surface flows into the Gulf at this station (Fig. 8). This relationship was inconsistent, however, as a second period of SW winds which began on July 23 did not have the same effect.

Currents and Sea Temperatures

Changes in sea temperatures at nearshore stations B and C were strongly coupled to currents (Fig. 3). At station B, temperatures at 21 m were coherent with alongshore currents at periods of 2.7 and 7.5 d. Phase leads indicated that currents led temperatures by approximately 110° (20 and 54 h at 2.7 and 7.5 d, respectively). Temperatures were also coherent with deeper onshore currents at periods >4 d. This coherence was virtually in phase, indicating that temperatures responded very quickly to changes in cross-shore currents.

The relationship between temperature and current at station C was similar to that observed at station B. Near surface temperatures were coherent with alongshore surface currents, and temperatures at the deep meter were coherent with cross-shore currents, at periods and phases similar to those at station B.

Temperatures at station A exhibited a solitary peak in coherence with cross-shore currents at periods of 3.5 d ($K = 0.84$, phase lead 60° or 14 h). Temperatures at 16 m were coherent and in phase with alongshore currents at a period of 1.8 d but not with longer period variations. Temperatures at greater depths (29 m) were coherent with both alongshore and cross-shore currents at periods of 3.5 d. Phase leads differed. Alongshore currents led temperatures by approximately 90° (21 h) whereas cross-shore currents followed temperature changes by 135° (32 h).

Discussion

The similarity of the temperature variations at periods >24 h over 150 km of coastline on the north shore of the Gulf of St. Lawrence suggests that the physical processes responsible for these changes operate over a large geographical area. The small phase leads, with west leading east by hours rather than days even at the longer periods, suggest that temperature variations are almost synchronous along this coast. The two major cooling events which occurred during July were both associated with strong periods of SW alongshore winds, NE alongshore currents, and the upwelling of cold water. Leggett et al. (1984) reported similar, large-scale synchronous coastal water temperature changes over much of the east coast of Newfoundland. These changes were due to coastal water mass exchanges driven by oscillations between the dominant SW (offshore) and less frequent (NE) onshore winds (Taggart and Leggett 1987).

Our data suggest that the SW alongshore winds which dominated this area during the period of study generated the observed NE alongshore currents. Under such conditions, within a range from shore up to the radius of deformation (4–8 km), Ekman drift would be expected to transport warmer surface waters seaward, to be replaced by cooler upwelling waters (Csanady 1982). NE winds would be expected to produce the opposite effect, with surface currents being SW and nearshore temperatures elevated following the onshore transport of warmer surface waters. These expectations are consistent with the coherences and phase leads observed between alongshore winds and nearshore sea temperatures, alongshore and cross-shore currents.

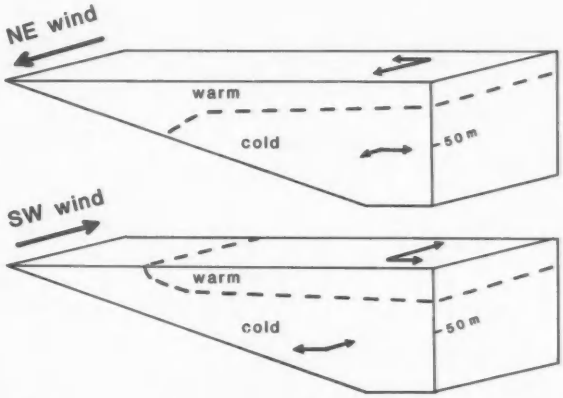

FIG. 14. Schematic diagram of nearshore hydrodynamics on the north shore of the Gulf of St. Lawrence forced by alongshore winds. Situations depicted are typical of summertime conditions characterized by dominant southwest winds (see text for greater detail).

We compared our results with the predictions of a simple two-layer model of pycnocline dynamics along a coastline (Csanady 1982, p. 100). We assume that the pycnocline and thermocline are at similar depths. Under this model, the thermocline is predicted to be forced to the surface when

$$I > h_t h_b^{-1} (h_t + h_b) f \cdot R_2$$

where I is the alongshore wind stress over time (square metres per second), h_t is the depth of the upper layer (metres), h_b is the depth of the lower layer (metres), f is the Coriolis parameter (per second), and R_2 is the radius of internal deformation (metres). We then examined the thermocline dynamics which occurred at Brador Bay between July 22 and 28, a period of rapid temperature decline nearshore (Fig. 7). We calculated the radius of deformation at this site to be 4200 m (Csanady 1982, p. 102). We calculated the minimum value of I for thermocline surfacing to be 5 m²/s. At the observed velocities of alongshore winds that occurred during this period, minimum values of I were exceeded after 20 h. This value is consistent with our observations of the thermocline dynamics, which indicate offshore movement of the thermocline in <1 d (Fig. 7). The lesser velocities of SW winds which began on July 10 generated impulses greater than 5 m²/s after 79 h. This range of time for the generation of thermocline movement (20–79 h) was consistent with the results of the spectral and regression analyses, which indicated that alongshore wind stress led nearshore temperature changes by 24–96 h.

Variations in nearshore sea conditions with shorter periods (<2 d) appear to be forced at least in part by cross-shore winds. Cross-shore winds were coherent at short periods (~1.8 d) with

sea temperatures independent of alongshore winds. At these higher frequencies, neither alongshore nor cross-shore winds were coherent with alongshore currents, which is inconsistent with alongshore forcing (Csanady 1982). At these higher frequencies, cross-shore winds were coherent with cross-shore currents. These observations are consistent with models of cross-shore wind-forced transport, wherein alongshore transport is cancelled by reverse geostrophic transport close to shore (Csanady 1982).

We conclude that the major observed variations in nearshore currents and sea temperatures were frequency-dependent responses to alongshore wind forcing (Fig. 14). Minor variations at higher frequency were forced by cross-shore winds and superimposed on the lower frequency variations. This conclusion is supported by evidence that directional forcing of sea levels on the north shore is frequency dependent (Garrett and Toulany 1982). These authors showed that sea levels (tidal frequencies removed) at Harrington Harbour, adjacent to Chevery, are coupled to alongshore and cross-shore winds at low frequency (period 8 d) but to cross-shore winds only at higher frequency (period 32 h).

The origin of the waters which intruded into the nearshore areas of the north shore can be inferred from the thermal and current structure of the Gulf. Cooler waters appear to have originated from the southwest where temperatures below 2°C were consistently observed at depths >70 m. These waters were likely at the boundary of the intermediate cold layer, within which temperatures below 0°C persist at depths below 100 m (Banks 1966). The greater intensity of temperature responses at the western end of the study area, where declines in water temperature to 0°C occurred, was likely related to its greater proximity to the cold waters of the Esquiman Channel.

The warmer waters observed in the nearshore area appear to have originated from the east. Waters on the Newfoundland side of the Gulf are several degrees warmer on average than those at Brador Bay (Dobson and Petrie 1984). Residual currents in the Gulf flow northward along the west coast of Newfoundland and counterclockwise at the northeastern limits of the Gulf (El-Sabh 1976). Under these conditions, northward currents would be expected to bring warmer waters from the south to the northwestern shores of Newfoundland. These waters may provide an input of heat into the northeastern Gulf in addition to that which results directly from atmospheric heating of surface waters. Under E wind conditions, these warmer waters are forced to the north shore.

We found no evidence during this study that inflow of waters from the Strait of Belle Isle affected the temperatures of Gulf waters. The inflow of pack ice which occurred during late June was exceptional and did temporarily depress temperatures. Inflow variability at other times appeared to be unrelated to sea temperature changes at any site monitored during this study.

Flow through the Strait of Belle Isle is highly variable, with periods of dominant flow in either or both directions (e.g. Toulany et al. 1987). Flow variability has been attributed to large-scale atmospheric pressure systems which lead to wind-forced alongshore currents which in turn cause sea level differences between the Gulf and the Labrador Sea (Garrett and Toulany 1981; Garrett and Petrie 1981). Under this model, differential sea level setups in the northern Gulf and Labrador Sea force directional flow in the Strait. Our data indicate that alongshore currents on the north shore are forced by alongshore winds. Although these winds were not significantly coherent with flows into the Gulf at station A, our data do indicate that periods of strong SW winds at times lead to flow changes toward the NE (out of the Gulf) and that periods of strong NE winds lead to flow changes toward the SW (into the Gulf).

Our observations lead us to hypothesize that the relative magnitudes of the influences of Labrador Sea waters and wind-driven upwelling of intermediate layer Gulf waters on the temperature declines along the north shore are inverse and part of a system regulated by larger scale pressure differences. Under this hypothesis, periods dominated by large-scale SW winds, such as occurred during this study and which typify summer wind conditions in the northern Gulf (Vigeant 1984), are accompanied by limited flows of Labrador current water into the Gulf. SW winds cause warmer surface waters to drift seaward from the north shore and hence upwelling conditions. NE winds of short duration cause warm surface Gulf waters to drift onto the north shore. The much less frequent periods dominated by large-scale NE winds are likely to be accompanied by increased flows of Labrador Sea waters through the Strait of Belle Isle. If such conditions persist, such as occurred during the period of ice flow into the Gulf in June 1985, temperature declines may occur on the north shore under NE winds.

The wind – sea temperature model we report is a first step in accounting for the temporal variability of biological events believed to be related to water temperature and coastal water mass advections over large areas of the north shore of the Gulf of St. Lawrence.

There is evidence that primary productivity is influenced by the demonstrated upwellings. Periodic anomolously high levels of nitrate, phosphate, silicate, and chlorophyll have been recorded along the north shore. Steven (1974) speculated that these resulted from localized nutrient inputs from sea bird excrement and the influx of Labrador Sea water. Our results demonstrate that the nearshore waters of this region experience episodic replacement by deeper waters from the intermediate layer. Nitrate levels in the intermediate layer of the Gulf are more than 10-fold those of surface waters (Steven 1974). We believe that the elevated levels of nutrients and productivity reported by Steven (1974) resulted from wind-forced upwellings.

At higher trophic levels, there is evidence of considerable energy transfer along the north shore. Here, as in other areas of Atlantic Canada, capelin migrate onshore during the summer to spawn. This concentration of pelagic fish inshore provides food for many species of migratory sea birds, whales, and piscivorous fishes (unpubl. data).

The efficiency of this energy transfer is regulated, in part, by coastal hydrodynamic conditions. For example, Atlantic cod migrate onshore to feed on spawning capelin on the St. Lawrence north shore, as they do in most areas of Atlantic Canada. Templeman (1966) hypothesized and Rose and Leggett (1988) demonstrated that nearshore movements of cod are regulated in part by wind-forced sea temperature variations.

It is likely that nearshore hydrodynamics also affect the migrations of other fishes. Like cod, mackerel migrate onshore seasonally on the north shore of the Gulf of St. Lawrence. Mackerel are pelagic and are usually found at temperatures above 7°C (Scott 1982; Bigelow and Schroeder 1953). We observed this range of temperatures only above the thermocline. We hypothesize that onshore movements of mackerel are associated with the landward advection of these warmer surface waters.

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Atmosphere–Ocean Coupling and Atlantic Cod Migrations: Effects of Wind-Forced Variations in Sea Temperatures and Currents on Nearshore Distributions and Catch Rates of *Gadus morhua*[1]

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Rose, G. A., and W. C. Leggett. 1988. Atmosphere–ocean coupling and Atlantic cod migrations: effects of wind-forced variations in sea temperatures and currents on nearshore distributions and catch rates of *Gadus morhua*. Can. J. Fish. Aquat. Sci. 45: 1234–1243.

Coastal upwellings and downwellings forced by alongshore winds on the north shore of the Gulf of St. Lawrence were associated with variability in onshore movements and inshore daily catches of Atlantic cod (*Gadus morhua*). Mean cod densities in the zone offshore from the trap sites, determined by hydroacoustic integration scaled by an in situ dual beam determined mean backscatter, ranged from 2.6 to 82.7 fish/10^5 m^3. Cod were located where sea temperatures ranged from −0.5 to 8.5°C. Catches were nil where temperatures were outside this range. Cod were located at depths which correlated with the strength of alongshore currents ($r = -0.55$). A path analysis model with predictors alongshore wind stress and current, sea temperature, and cod density accounted for 76% of the variance in catch. Cod abundance in the offshore zone accounted for the most variance independent of other factors (27%). Sea temperature and currents accounted for 15 and 13%, respectively. Peaks in catch (to 1500 kg/d) coincided with maximum rates of decline in sea temperatures and with NE upwelling currents. Low catches coincided with maximum rates of increase in temperatures and with SW currents. In a regression model, four lags of alongshore wind accounted for 83% of the variance in catch.

Les auteurs ont établi une relation entre les courants côtiers ascendants et descendants provoqués par les vents parallèles à la rive de la côte nord du golfe du Saint-Laurent et la variabilité des déplacements vers la côte et des prises côtières quotidiennes de morue de l'Atlantique (*Gadus morhua*). La densité moyenne de morues dans la zone située au large des filets-trappes, déterminée par intégration hydroacoustique étalonnée par réflexion moyenne d'un faisceau double *in situ*, variait de 2,6 à 82,7 poissons/10^5 m^3. Les morues étaient décelées dans les endroits où la température de l'eau se situait entre −0,5 et 8,5°C. Aucune prise n'a été effectuée aux endroits où la température débordait de cette gamme. Les morues étaient trouvées à des profondeurs corrélées avec la force des courants parallèles à la côte ($r = -0{,}55$). Un modèle d'analyse par cheminement à paramètres de prévision pour la contrainte du vent et des courants, la température de la mer et la densité des morues le long de la côte a permis d'expliquer 76 % de la variance des prises. L'abondance des morues dans la zone hauturière expliquait la plus grande partie de la variance indépendante des autres facteurs (27 %). La température de la mer et les courants en représentaient 15 et 13 % respectivement. Les prises maximales (jusqu'à 1 500 kg/d) coïncidaient avec les taux maximum du déclin de la température de la mer et les courants ascendants NE. Les faibles prises coïncidaient avec les taux maximum de l'augmentation de la température et les courants SW. L'utilisation d'un modèle de régression a montré que quatre décalages du vent le long des côtes expliquaient 83 % de la variance des prises.

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Seasonal onshore migrations of Atlantic cod (*Gadus morhua*) sustain the inshore fishery for this species in Atlantic Canada. The magnitude, timing, and duration of these migrations are highly variable at time scales ranging from days to years. This variability is directly reflected in the variable successes of the inshore fishery for cod (Lear et al. 1986). Coastal water mass advections, and the sea temperature variability that results, have long been hypothesized to be major regulators of the short to medium period (days to weeks) variability in cod movements and catches (Jeffers 1931; McKenzie 1934; Lee 1952).

Templeman (1966) theorized that the onshore movements of cod to northeastern Newfoundland and Labrador were associated with coastal hydrodynamics and that this partially regulated the success of the nearshore cod trap fisheries there (Fig. 1). Templeman suggested that after cod ascended from their deepwater winter ranges, to depths and temperatures above those of the Labrador current, their movements followed the depth oscillations of the thermocline. He proposed that during periods of offshore winds, warm inshore surface waters would be transported seaward, to be replaced by cooler subthermocline waters having temperatures within the range favorable to

[1]Contribution to the program of GIROQ (Groupe Interuniversitaire de Recherche Océanographique du Québec).

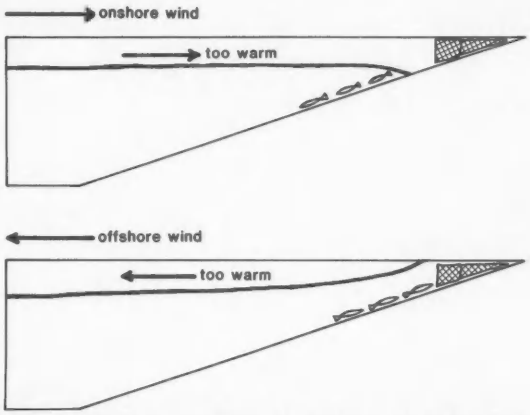

FIG. 1. Schematic representation of Templeman's (1966) hypothesis regarding the relationship between coastal water mass dynamics and cod catch.

cod. Under these conditions, shoreward movements of cod would be uninhibited by thermal barriers and catches would be high. During periods of onshore winds, surface layer waters having temperatures unfavorable to cod would be transported shoreward. When such waters reached the depths of the shallow traps (15–30 m), cod would be isolated from this fixed gear by thermal barriers, and catches would be low.

Several attempts to test Templeman's theory have been made. The results have been inconclusive, largely because cod distribution and nearshore hydrodynamics (not simply temperature) were not simultaneously assessed (Buchanan et al. 1982; Lear et al. 1986; Taggart and Frank 1987). The shortcomings of these studies reflect the difficulty of accurately assessing fish abundance and distribution at relatively small scales and the historic failure to incorporate physical oceanographic studies into the design and execution of studies of fish migration (Leggett 1977, 1984).

In this paper we test Templeman's (1966) central hypothesis: that variations in nearshore sea temperatures, caused by coastal water mass advections, regulate the variability in the catch rates of the nearshore trap fishery. We also test two predictions arising from that theory: (1) that the distribution of cod of commercial size is limited by extremes of temperature and (2) that wind-forced incursions of waters having temperatures favorable to cod into the nearshore zone are correlated with movements of cod into shallower waters.

Methods

The study was carried out at Brador Bay, Quebec, in the northeastern corner of the Gulf of St. Lawrence (Fig. 2). The study area was approximately 250 km² (approximately 30 km of coastline by 5–8 km offshore). Maximum water depth was approximately 100 m. In this report, nearshore refers to depths <30 m (approximately equal to the 10-fathom isobath, Fig. 2) and corresponds to the area where cod traps were located. Offshore refers to the balance of the study area. The Brador Bay area has supported an active inshore fishery for several hundred years. It was here at Bonne Esperance that the cod trap was developed and first used (Whiteley 1977). Several of the trap sites monitored in this study have been used for nearly 100 yr. The success of this trap fishery has always been highly variable; the fishery typically lasts 4–5 wk, starting during the last week of June (L. Dumas, President, Lower North Shore Fishermen's Association, Lourdes-de-Blanc-Sablon, Que., pers. comm.). On the basis of its history, we judged this fishery to typify the trap fishery for cod in Atlantic Canada and thus to be ideal for testing theory relating to variability in trap catch rates.

We monitored sea temperatures, currents, meteorological conditions, cod distribution and abundances, and trap catches of cod at Brador Bay during July and August 1985. The meteorological and oceanographic data used, a description of their analyses, and of the wind-forced hydrodynamics that occurred during this study, are reported in a companion paper (Rose and Leggett 1988b). From these data, daily means were calculated to match the sampling frequency of the biological data. Winds (metres per second) and wind stresses are identified by their direction of origin and currents (metres per second) by their direction of travel. Wind speeds were converted to wind stress:

$$t_y = 0.000002W \cdot W_y$$

where t_y = wind stress along axis y (alongshore or cross-shore) (square metres per square second), W = wind speed (<7) (metres per second), and W_y = component of wind vector along axis y (metres per second) (Csanady 1982).

The distribution and abundance of cod in the study area were assessed daily from July 11 to August 2, 1985, using a Biosonics model 105 echo sounder (120 kHz), fitted with a dual-beam transducer (10 and 25°) mounted in a v-fin towing body. Pulses of 0.8 ms width were generated at a rate of 5/s. A Biosonics tape recorder interface and Sony video recording system stored all data. The transducer was towed beside the 9-m boat at a depth of approximately 2 m. Transducer stability was enhanced by a series of movement-damping springs which isolated the movements of the boat from that of the towed body.

An average of 43 (range 21–66) acoustic transects of 1 km length were run daily. All transects were run during the daylight hours between 0600 and 1600. Transects were linked systematically to form zigzags designed to sample all spatial and depth ranges of the study area. Precise transect bearings were chosen randomly. To counter problems of serial correlation in the transect data, the cluster sampling approach was used (Williamson 1982). Boat speed during all transects was 10 km/h.

Relative estimates of density were obtained by the standard techniques of echo integration (Burczynski 1982), using a Biosonics model 120 digital echo integrator. This integrator samples and digitizes input voltages every 134.2 μs (0.1 m). Integration of digitized 20 log R amplified voltages was carried out according to the following formula:

$$D_{xt} = (V_i)_x^2 \cdot P^{-1} \cdot N_x^{-1} \cdot K_t \cdot K_s$$

where D_{xt} = acoustic target density for the depth interval x (metres) and transect t, $(V_i)_x^2$ = squared sampled voltage for increment i in interval x, N_x = number of 0.1-m increments in interval x, P = total number of echo sounder pings in each transect (constant), K_t = a constant incorporating an empirically determined correction of the 20 log R + 2α R time varied amplifier gain for each depth interval x (α = absorption coefficient for sound in sea water of given density (decibels per metre), and K_S = a constant determined by system parameters. Integrator strata (D_{xt}) were blocks with length of 1 km and depths of 10 m. A bottom interval of 1 m was not sampled to prevent integration of bottom echoes. Cod were discriminated from capelin (*Mallotus villosus*) and mackerel (*Scomber scom-*

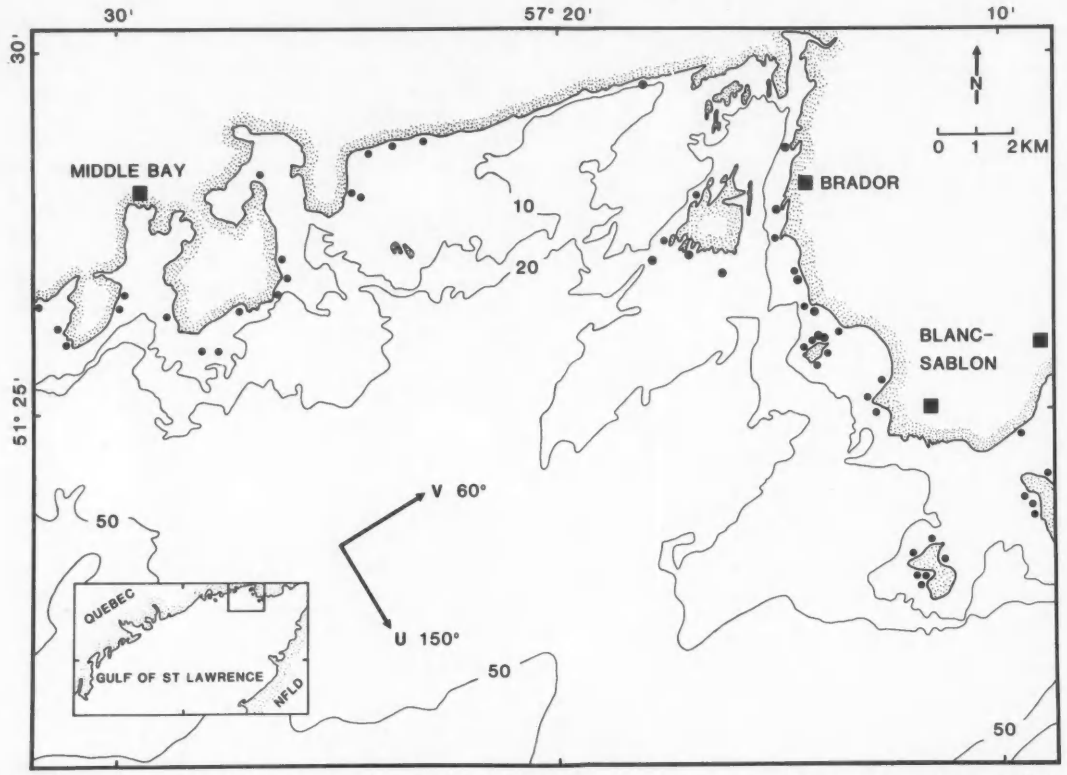

FIG. 2. Brador Bay study area showing locations of traps and directions of the *u* and *v* components for winds and currents. Isobaths in fathoms (1 fathom = 1.8 m).

brus) by employing the quadratic discriminant functions developed by Rose and Leggett (1988a).

Relative density estimates were scaled to absolute values (fish per 10^5 m^3) with an estimate of the mean backscattering cross section of cod measured at Brador Bay (0.002973 m^2, mean target strength (decibels) = -31.4, $n = 1576$) (Rose and Leggett 1988a).

A total of 52 cod traps were monitored, in cooperation with their owners, for the duration of the 1985 trap fishing season (July 2 to August 3) (Fig. 2). Each trap occupied a fixed site licensed by the Department of Fisheries and Oceans. The location, depth, and dimensions of each trap were recorded at the start of the fishing season. Trap depths ranged from 13 to 26 m. Each day, the catches of an average of 20 (range 5–30) randomly chosen traps were assessed. The lower sample sizes occurred at the beginning and ends of the trap season, when the total number of traps being fished was less than during the central part of the fishing season. Each catch represents one trap-day of effort, with the exception of a few data (<4% of total). For these exceptions, when a trap had not been checked daily, the catch represented more than 1 d of effort. These catches were averaged over the period of effort. Catches visually estimated by the fishermen to be more than 100 kg were removed from the trap and weighed. Catches estimated to be less than 100 kg were not removed from the trap; in these cases the fisherman's estimate of catch weight was recorded. Weighed catches which included visually estimated catches from previous days were reduced by the amount equivalent to the sum of the estimated catches. Tests of the ability of fishermen to assess weights of trap catch (their predictions versus actual weights of lifted catches) indicated that their estimates were seldom inaccurate by more than 10%.

For comparison with physical variables, 3-d moving averages of daily catch rates and cod densities were calculated to reduce high-frequency variability. This averaging made comparisons between catch and the physical variables meaningful. Comparisons of trap depth temperatures indicated that high-frequency (1 to approximately 2.5 d) variability was incoherent except at adjacent sites (Rose and Leggett 1988b). Hence, an explanation of high-frequency variability in trap catch, based on physical factors, must assess smaller spatial scales (by trap or group of adjacent traps) than we do here. All meteorological and oceanographic variables were also filtered to reduce high-frequency variability (for details see Rose and Leggett 1988b).

Many colinearities were evident among the various physical and biological variables measured. This colinearity precluded the use of the common multivariate techniques (i.e. multiple regression) to assess the individual contributions of predictor (independent) variables to the variance of catch and made their simple correlations with the dependent variable difficult to interpret.

We used a path analysis (Sokal and Rohlf 1981, p. 642) to test Templeman's theory. Path analysis is a modified form of multivariate regression which enables the assessment of the contributions of colinear independent variables to the explained variance. Path analyses are used to model alternative structural

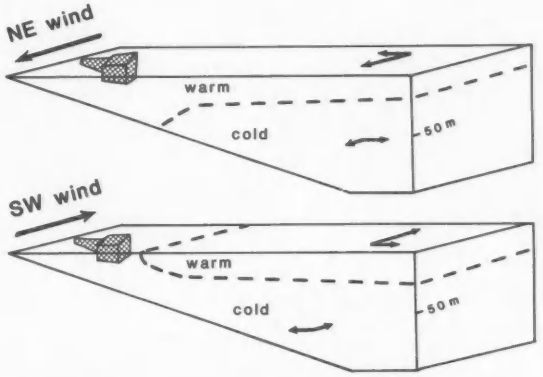

FIG. 3. Schematic representation of atmosphere–ocean coupling at Brador Bay, 1985. Arrows indicate magnitude of alongshore and cross-shore components of current under SW and NE alongshore wind conditions.

relationships of cause and effect among variables and not to assess statistical significance. The fit of a path model is judged by the degree to which the sum of the magnitudes of the pathways between predictor (independent) and criterion (dependent) variables duplicate the simple correlation between them. The assumptions of path analysis are similar to those of standard multiple regression.

Prior to entry into the model, two types of data transformation were used. Variables that contained a linear trend (sea temperature and catch) were detrended. All variables were made stationary (constant mean and variance) by single period differencing (Fuller 1976). Thus transformed, all variables were free of significant autocorrelation and had distributions which did not differ significantly from normalcy (Kolmorogov–Smirnov test).

Statistical analyses were carried out on SAS/STAT for personal computers (SAS Institute, Cary, NC), SYSTAT (Systat Inc., Evanston, IL), and STATGRAPHICS (Statistical Graphics Corp., Rockville, MD). Spectral analyses were carried out on programs written in regression analysis of time series (RATS) language (VAR Econometrics, Evanston, IL), according to methods outlined in Rose and Leggett (1988b). Statistical significance is indicated if $P < 0.05$.

Results

Winds, Currents, and Temperatures

A detailed description of the relationships among winds, currents, and sea temperatures in the study area, and along 150 km of coastline between La Tabatiere and Blanc-Sablon, Quebec, on the north shore of the Gulf of St. Lawrence, is presented in Rose and Leggett (1988b). Here we present only the findings of that analysis pertinent to the present study.

Longer period temperature variations (>3 d) over 150 km of the lower north shore were virtually synchronous although west led east at the longest of periods (>7 d). Shorter period temperatures variations (<2.5 d) were coherent only at adjacent sites.

Currents and sea temperatures in Brador Bay (tidal variations removed) were coherent with alongshore wind stress at periods >2 d, and with cross-shore wind stress at shorter periods. In 1985, the winds in this area were predominantly from the SW

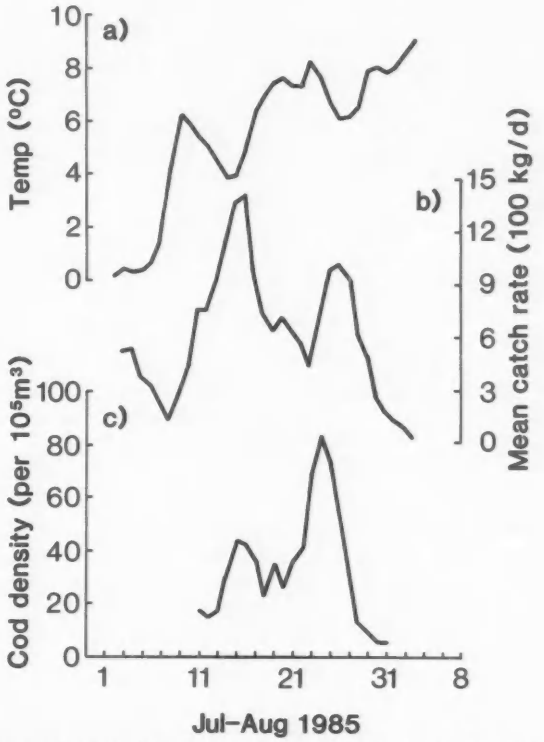

FIG. 4. (a) Mean daily sea temperature at trap depth, (b) mean daily catch per cod trap, and (c) average cod densities within the study area during the 1985 trap fishery for cod at Brador Bay.

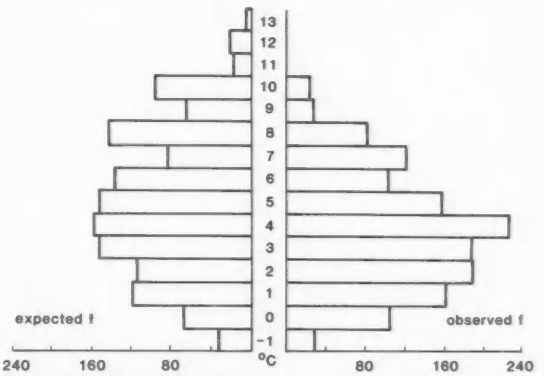

FIG. 5. Observed and expected frequency of occurrence of cod over the range of sea temperatures encountered. Negligible cod signals (equivalent to <1 average fish/10^5 m^3) were discounted. Expected values were based on the assumption of a random distribution of cod in relation to temperatures sampled. Observed frequencies differed significantly from expected frequencies ($\chi^2 = 735$, df = 14, $P < 0.05$).

(alongshore) with occasional NE winds. This is the typical summertime condition in eastern Canada (Vigeant 1984; Taggart and Leggett 1987).

Alongshore SW wind stress (direction from) forced alongshore NE currents (direction to) to depths of at least 36 m within a single radius of deformation of shore (4200 m at Brador Bay) (Rose and Leggett 1988b). Within this zone, the currents

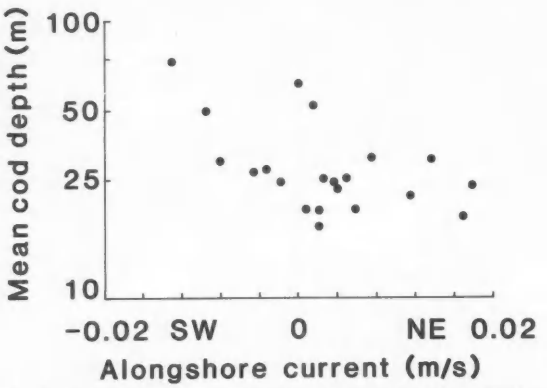

FIG. 6. Relationship between alongshore currents and mean depth at which cod were surveyed (depth (m) = 35 − 1047 current (m/s), $r = -0.55$, $P < 0.05$). Currents are daily means of deep meters at stations B and C of Rose and Leggett (1988b). Currents travelling NE (+) and SW (−).

generated under SW wind conditions contained seaward components at the surface but landward components near the bottom (Fig. 3). This upwelling caused thermocline elevation and declines in nearshore sea temperatures within 24 h. The alongshore SW wind stresses which occurred during the study period generated sufficient impulse to force the thermocline to the surface in as little as 20 h (Csanady 1982; Rose and Leggett 1988b). NE wind stress generated the opposite effects, as downwelling warm surface waters were forced onshore. These dynamics are consistent with alongshore forced Ekman transport (Csanady 1982; Rose and Leggett 1988b).

Higher frequency (0.5–1/d) nearshore temperature variations were related in part to cross-shore wind stress. Offshore NW wind stress generated offshore SE surface currents and onshore NW deeper currents. Onshore SE wind stress led to the opposite conditions. No alongshore currents were generated and temperature variability was low at these frequencies. These observations were consistent with models of cross-shore transport (Csanady 1982; Rose and Leggett 1988b). We do not deal extensively with these smaller scale effects here because the maximum resolvable frequency of our cod abundance and catch data is 0.5/d.

Cod Abundance and Distribution

Mean densities of cod in the study area ranged from 2.6 to 82.7 fish/10^5 m^3 (Fig. 4c). Two main peaks in cod density occurred, the first from July 13 to 17 and the second from July 23 to 26. These peaks coincided approximately with peaks in catch. Cod densities were significantly and positively correlated with trap catch ($r = 0.61$, $P < 0.05$), but changes in catch were not proportional to changes in cod abundance as determined hydroacoustically. During SW alongshore winds, when upwelling conditions dominated the nearshore area, cod densities increased in the study area. Mean daily cod densities were negatively correlated with alongshore winds from the NE (+) (not wind stress) ($r = -0.49$, $P < 0.05$).

During acoustic sampling, more than 95% of the cod surveyed were located in waters having temperatures within the range of −0.5 to 8.5°C ($n = 4450$) (Fig. 5). The frequency of occurrence of cod in relation to sea temperature differed significantly from that expected under the hypothesis that cod

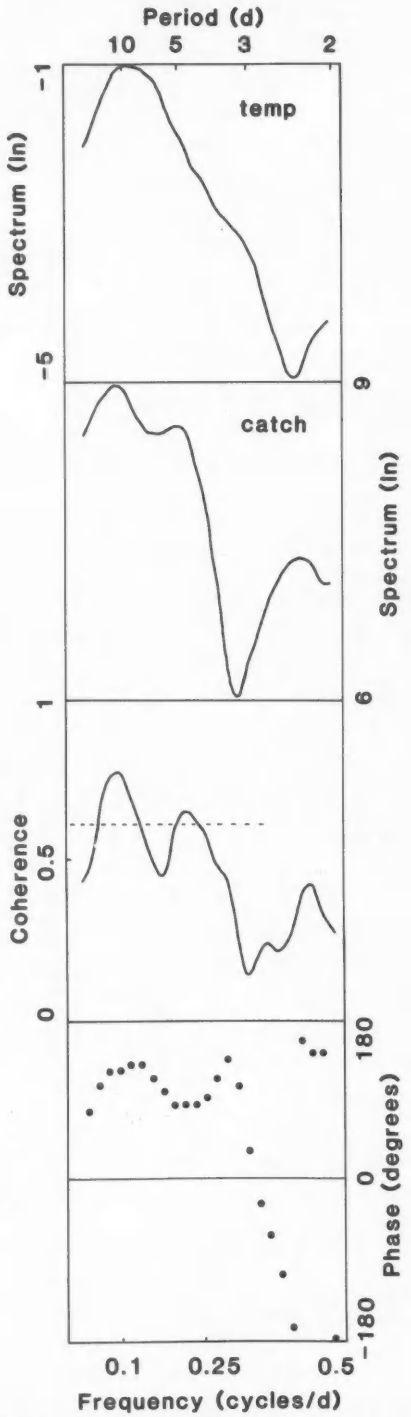

FIG. 7. Spectra, coherence, and phase leads between mean daily cod catch and mean daily sea temperature at trap depth during the 1985 fishery at Brador Bay. Line across coherence plot indicates coherence > 0 ($P < 0.05$). Confidence limits (95%) for phase spectrum are ±20° at periods of 10 d and ±25° at periods of 4–5 d (Jenkins and Watts 1968).

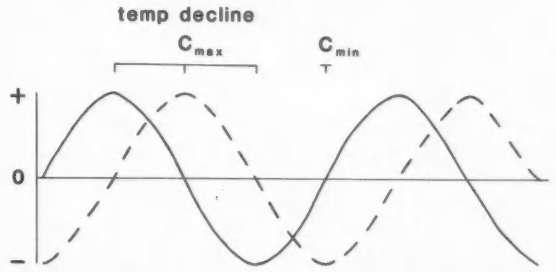

FIG. 8. Schematic diagram representing relationship between temperature (solid line) and cod catches (broken line) which are 90° out of phase (temperature leading). C_{max} and C_{min} are maximum and minimum catches.

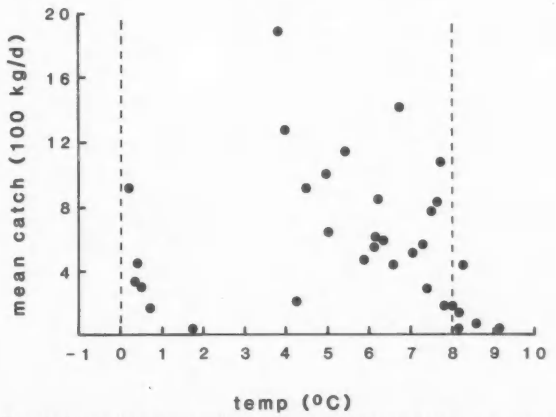

FIG. 9. Mean daily trap catches and mean daily sea temperature at trap depth during the 1985 trap fishery at Brador Bay.

would be randomly distributed in relation to sampling effort ($\chi^2 = 735$, df = 14, $P < 0.05$). In general, cod occurred at frequencies greater than expected within the range of sea temperatures of 0.5–4.5°C and at frequencies equal to or less than expected outside this range.

Cod were located within 10 m of the bottom in almost all cases during this study (July and August). We observed one instance of cod feeding near the surface during the daytime under conditions of heavy fog. The average depth at which cod were distributed was related to the strength and direction of the alongshore current. When bottom alongshore currents were NE (+), cod were located on average in shallower waters than when these currents were SW (−); alongshore currents were negatively correlated with the mean depth at which cod were surveyed ($r = -0.55$, $P < 0.05$) (Fig. 6). It is evident that when currents were strongly toward the NE cod did not on average locate themselves at depths of <20 m.

Daily Trap Catches

Mean daily catch of individual traps ranged from near zero to 1500 kg/d over the fishing season (Fig. 4b). Two main peaks in catch occurred, the first from July 11 to 16 and the second from July 24 to 27. These peaks coincided with decreases in sea temperature at the depths of the traps (Fig. 4a).

Trap catches were coherent with nearshore sea temperatures at periods of approximately 5 and 10 d (Fig. 7). Power spectra indicated that for both catch and temperature, peak variance was

TABLE 1. Statistics from the path analysis model relating changes in mean daily trap catch (kg/d) at Brador Bay, 1985, to trap depth sea temperature (°C), alongshore current (m/s), alongshore wind stress (m^2/s^2), and cod density in Brador Bay ($fish/10^5 m^3$). All variables are single period differences (Fuller 1976). Wind is lagged 2 d prior to catch day; all other variables have no lag.

Pearson correlation matrix					
	Catch	Temp	Current	Wind	Cod
Catch	1.00	−0.50	0.62	−0.51	0.61
Temp		1.00	−0.27	0.58	0.03
Current			1.00	−0.48	0.27
Wind				1.00	−0.13
Cod					1.00

Standard partial regression coefficients (path coefficients) of catch vs.:	
Cod	0.52
Temp	−0.39
Current	0.35
Wind	−0.05

Breakdown of variance of path analysis model (%)		
Single variables	Cod	26.7
	Temp	15.4
	Current	12.5
	Wind	0.0
Colinear paths	Current–cod	9.8
	Current–temp	7.4
	Wind–temp	2.3
	Wind–current	1.7
Total accounted for		75.8
Error		24.2
Total		100.0

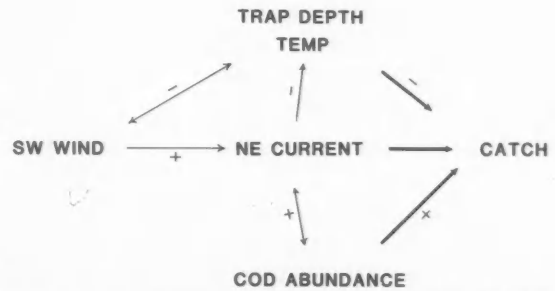

FIG. 10. Schematic diagram representing the path analysis model relating changes in mean daily trap catch (kg/d) at Brador Bay, 1985, to trap depth sea temperature (°C), alongshore current (m/s), alongshore wind stress (m^2/s^2), and cod abundance ($fish/10^5 m^3$). Heavy arrows represent standardized partial regression coefficients which assess effects independent of other factors; light arrows represent simple correlation coefficients. Single arrowheads imply cause and effect; double arrowheads represent simply correlation. Signs of relationships are indicated.

at periods of 10 d. Catch exhibited a peak in variance between 4 and 5 d that was not evident in the temperature series. Phase leads indicated that temperature led catch by values not significantly different from 90° at the coherent periods of 5 and 10 d (Fig. 7). Therefore, temperature declines coincided with above-

TABLE 2. Statistics of a multiple regression model of daily changes in mean trap catch (kg/d) as a function of the mean daily alongshore component of wind vector (m/s) lagged 0–4 d (i.e. wind4 = wind 4 d prior to day of catch). All values are single period differences (Fuller 1976).

Dependent variable: daily trap catch, $R^2 = 0.83$ (adjusted for df)			
Predictor	Coefficient	t	P
Constant	−48.28	−2.46	0.03
Wind0	−83.15	−5.81	0.01
Wind1	−47.55	−3.33	0.01
Wind2	−23.95	−1.72	0.10
Wind3	0.65	0.04	0.96
Wind4	−14.38	−0.98	0.34

ANOVA for full regression

Source	Sum of squares	df	Mean square	F	P
Model	464 785	5	92 957	15.2	0.001
Error	61 170	10	6 117		

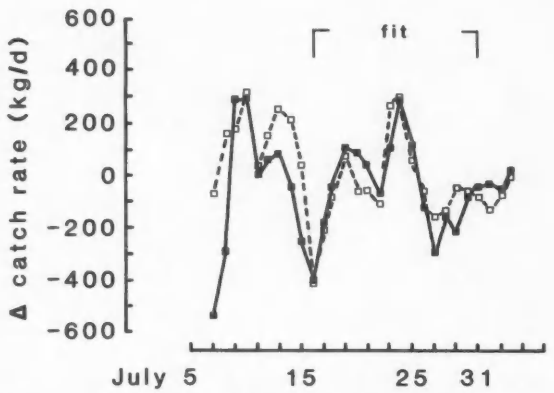

FIG. 11. Fitted values of alongshore wind model of catch (July 16–31), predicted values of catch for periods prior to July 16 and after July 31 (solid squares and line), and actual catches (open squares, broken line) for the trap fishery for cod at Brador Bay during 1985. Catch values are single period differences and thus represent change in catch from the previous day (Fuller 1976). Details of this model are given in Table 2.

average catches; catches peaked when negative changes in temperature were greatest and were least when temperatures increased at the maximum rate (Fig. 8).

Trap catches approached zero when sea temperatures at trap depth approached 8.5°C (Fig. 9). At sea temperatures between 0 and 1°C, catches were average. At temperatures <0°C, there was no fishing effort. Within the range of sea temperatures of 0–8.5°C, no pattern of catch in relation to temperature was discernable. Very few catches were landed at temperatures between 1 and 4°C because waters warmed through this range very quickly (Fig. 4a).

The path analysis model whose structure most closely duplicated the correlations among the predictor variables alongshore wind stress, depth-averaged alongshore current, sea temperature at trap depth, and cod abundance within the study area accounted for 76% of the variance in mean daily trap catch (Table 1). We used alongshore wind stress 2 d in advance of the other variables because this lag had the highest correlation with both currents and sea temperatures on the day of the catch. Inclusion of additional lags of wind stress increased the amount of variance explained but did not aid our understanding of this system and were therefore omitted. This model infers that alongshore wind stress forces alongshore currents which in turn induce changes in nearshore sea temperatures and cod abundances (Fig. 10). Of the univariate paths examined, those of cod abundance offshore, trap depth sea temperature, and alongshore currents had the greatest influence on trap catch, accounting for 26.7, 15.4, and 12.5% of the variance, respectively. Bivariate paths explained an additional 22% of the variance. The effects of wind stress were limited to regulation of currents and sea temperature. Currents were weakly correlated with cod abundance in the study area.

A multiple regression model employing the alongshore component of wind vectors (not wind stress) up to 4 d prior to the day of the catch as the independent variable explained 83% of the variance in cod catch during the period for which hydroacoustic surveys were carried out (the period covered by the path model) (Table 2). In this model we used wind rather than wind stress because it was a slightly superior predictor of catch. We included wind lagged up to 4 d in the model, even though lags of 3 and 4 d accounted for only small amounts of the explained variance, because we had shown previously that for longer time series, alongshore winds have predictive power 4 d in advance of nearshore sea temperatures (Rose and Leggett 1988b). Inclusion of cross-shore winds as predictors in the model did not increase the variance explained.

We used the alongshore wind model to forecast and backcast daily changes in catch during the initial and terminal periods of the 1985 trap fishing season when hydroacoustic estimates of fish abundance were not available. For these periods, daily changes in catch rate predicted by the wind model and those observed were significantly correlated ($r = 0.85$, $P < 0.05$). Oscillations in predicted and observed catch were synchronous and had maximum variance at periods centred around 5 and 10 d (Fig. 11).

Discussion

The first prediction of Templeman's (1966) theory, that the distribution of cod of commercial size is delimited by extremes of water temperature, was supported by our hydroacoustic assessments of cod distribution. Cod were rarely found at temperatures outside the range −0.5–8.5°C. Both higher and lower temperatures were available to cod and were repeatedly surveyed during this study. This is strong evidence that cod of commercial size avoided waters with temperatures outside this range. There have been many reports of temperatures at which cod were caught, but few in which sampling effort was exerted randomly or in relation to the occurrence of the temperature field available to the fish. Reports based on sampling over a wide range of temperatures are supportive of a −0.5–8.5°C temperature range for northwest Atlantic cod during the summer period (June–August). During the winter months, cod have been shown to be less tolerant of low temperatures (Woodhead and Woodhead 1959) and are only rarely found where sea temperatures are outside the range 1–4°C (Martin and Jean 1964). In the summer of 1900, Labrador cod were jigged in waters having temperatures within the range −0.3–8.2°C (Grenfell 1909). Jean (1964) reported that cod were seldom caught in waters with bottom temperatures outside the range −0.5–8°C in the Gulf of St. Lawrence and outside the range 1–8°C on the Scotian Shelf in May and June. Templeman and May (1965)

reported that Labrador Sea cod were caught inshore in waters with temperatures of −0.8–8.7°C in summer. Scott (1982) found that cod were only rarely located outside the temperature range 3–7°C on the Scotian Shelf. Cod undoubtedly test and at times traverse these limits of temperature tolerance, especially during migratory feeding forays (Lee 1952; Templeman and Fleming 1956). It is doubtful, however, that cod >40 cm in length withstand temperatures outside the range −0.5–8.5°C for extended periods. A physiological basis for the lower limit was suggested by Woodhead and Woodhead (1959), who indicated that during the July–September period, cod showed physiological imbalance at sea temperatures below −0.5°C. Cod do not produce antifreeze glycopeptides in response to photoperiod as do species that are better adapted to survival at subzero temperatures (Fletcher et al. 1987). Although cod can acclimate to subzero temperatures, they do so slowly and inefficiently (Fletcher et al. 1987). Smaller cod can and do exceed these limits (Templeman and Fleming 1965). We found apparently healthy 15–20 cm cod in waters near −1°C (unpubl. data).

The range of temperatures at which cod are most frequently caught has been reported to differ among stocks (Jean 1964; Scott 1982). It remains unclear if differences among stocks are genetically based or simply the result of geographically variable temperature availability. Brador Bay is an area of potential overlap in the summer ranges of the Labrador Sea and northern Gulf cod stocks (Templeman 1974; Lear 1984). We therefore conducted a stock identification study to identify the stock under study. Vertebral counts and the criteria given in Templeman (1981) were used to assign fish to a stock: northern Gulf stock mean vertebral number <54.1; Labrador stock mean vertebral number >54.7. A total of 342 cod were sampled on 16 d from July 9 to August 10, 1985. These fish averaged 52.5 cm in length (FL, range 43–67 cm). The mean number of vertebrae was 53.5 (range 48–58). Mean counts did not vary significantly among samples taken over time. All daily means, even those with very low n (range of daily n = 4–70), classified as northern Gulf cod. Further details of the meristics are given in Langevin (1986). We conclude that the fish present in Brador Bay during this study were predominantly, if not exclusively, of the northern Gulf stock.

The second prediction of Templeman's (1966) theory, that movements of cod into shallower waters are correlated with landward incursions of waters having favorable temperatures, was also supported by our hydroacoustic surveys of cod distribution. During periods of NE currents, when the cooler subthermocline waters were transported landward following Eckman drift of surface waters seaward, cod were located inshore in shallower waters. In contrast, during periods of SW currents, when heated surface waters were transported landward and the thermocline was depressed, cod moved offshore to deeper waters. These alongshore NE and SW currents were forced by alongshore winds (Rose and Leggett 1988b). Movements of cod into shallower/deeper waters thus followed SW/NE winds.

The path analysis model suggests that the direct effects of the abundance of cod offshore from the trap sites, nearshore sea temperatures, and alongshore currents are the chief regulators of trap catch at the scales considered here. This result supports the general predictions of Templeman's (1966) theory, but suggests that at least on the north shore of the Gulf of St. Lawrence, the hydrodynamic system is forced by alongshore winds, and not by cross-shore winds as Templeman proposed. Within the study area (smaller scales), spatial variability in catch and cod abundances occurred which could not be explained by this model. We believe this variability, and the residual variance in the model, to be directly attributable to spatial variability in the physical variables and in capelin abundances. Further work is planned to test these ideas.

Cod abundance in the zone offshore of the trap sites had the greatest independent influence on catch. This is not surprising. It is perhaps more noteworthy that cod abundances in Brador Bay can account for only 36% of the variance in daily trap catch (27% if considered independent of other factors). It is evident that the success of the fishery is not a simple function of stock size, even at the relatively small scales measured here, but results from the complex interactions of fish abundance, hydrodynamics, fish behavior, and the methods of the fishery.

Two lines of evidence suggest that cod catches were related to thermocline dynamics as proposed by Templeman (1966). This evidence comes from the spectral and path analyses. The results of spectral analysis between sea temperatures at trap depth and catch rates indicate that catches peaked when the rate of temperature decline was maximum and were lowest when temperature increases were greatest (Fig. 7). Temperature changes are greatest at the depths of the thermocline. Depressions and elevations of the thermocline in relation to trap depth should therefore induce the largest positive and negative rates of change in temperatures there. Hence our data suggest that catches were highest immediately following the elevation of the thermocline above trap depth and were lowest following depression of the thermocline to depths below the traps. Catches remained above average for the duration of the temperature decline.

The path model confirmed that declines in sea temperature at the depth of the traps led to increases in catch. It is important to note that within the temperature range 0–8°C, catches varied in relation to temperature changes but not in relation to temperature per se.

Our data also suggest that currents influence cod distributions and movements, and hence catches, independent of temperature. The path model coefficient between alongshore current and cod catch was almost as large for currents as for temperatures (Table 1). This relationship may reflect the tendency of fish to maintain position at specific temperatures within a water mass. Cod may also use currents and local water movements to facilitate migration. Trout (1957) proposed that Spitsbergen cod moved somewhat passively within a defined water mass. Echosounding studies on the same stock showed that movements of cod were closely associated with variations in the strength of the locally dominant Spitsbergen current (Beverton and Lee 1965). We believe the coastal movements of cod in relation to water mass movements demonstrated in Brador Bay to be but a special case of the general tendency for cod to migrate in concert with hydrographic regimes at many scales.

It is probable that cod movements were responsive to the coastal oceanographic system of the north shore (Rose and Leggett 1988b) at scales larger than that of our study area. It is evident that cod moved into and out of the study area during the period of study. The study area was not a closed system. We believe that the area we surveyed and its related physical and biological interactions represent only a part of a much larger system. The extent of this system, and the total scale of the demonstrated cod movements, both in the alongshore and cross-shore directions, are not known. We do know, however, that the physical system of coastal dynamics with which the movements of cod were strongly associated occurred over at least 150 km of coastline and that upwelling and downwelling con-

ditions wre virtually synchronous over that area (Rose and Leggett 1988b).

The multiple regression model employing lags of alongshore wind as predictors accounted for 83% of the variance in daily changes in catch. The lag structure of the wind model was similar to, and partially based on, wind models of nearshore sea temperatures (Rose and Leggett 1988b). In the models of nearshore sea temperature and cod catch, alongshore winds had predictive power up to 4 d prior to temperature and catch. We conclude that the synchronous oscillations in nearshore sea temperature and trap catch were both responses to alongshore winds. The high correlation between observed catches and the forecasted and backcasted predictions of catch derived from the wind model strongly supports the view that the wind-forced system upon which this model was based, and its regulation of cod catch, was operative beyond the time range on which the model was based.

We can only speculate on why wind predicts catch somewhat more effectively than does wind stress. In accordance with theoretical considerations (Csanady 1982), wind stress was more coherent with currents in the study area than was wind (Rose and Leggett 1988b). However, wind was more coherent with nearshore sea temperatures than was wind stress at several frequencies and was more highly correlated with cod abundance than was wind stress. These are probably the bases for the superior predictions of catch from wind.

We believe that nearshore currents and temperatures are among the chief regulators of trap catch not only on the north shore of the Gulf of St. Lawrence but in most areas of Atlantic Canada. We expect the factors that regulate nearshore temperatures and currents to be scale and site dependent. A comparison of the north shore of the Gulf of St. Lawrence and the northeastern coast of Newfoundland is illustrative. These two regions are subject to similar SW/NE winds (Taggart and Leggett 1987; Rose and Leggett 1988b), but the average orientation of the wind relative to the coastline differs by approximately 90°. Prevailing SW winds are on average alongshore on the north shore of the Gulf of St. Lawrence but offshore in northeastern Newfoundland. Templeman (1966) hypothesized that these offshore winds along the coast of eastern Newfoundland generated upwelling conditions favorable to successful trap fishing there. The importance of cross-shore winds as regulators of nearshore hydrodynamics in small bays in that area and their influence on biological phenomena have subsequently been confirmed (Frank and Leggett 1982; Taggart and Leggett 1987).

It is evident, however, that the prevailing SW/NE winds which occur here may be alongshore at intermediate scales. Much of the coastline of eastern Newfoundland, including the Atlantic Avalon coast and the along-bay axes of several large bays (Conception, Trinity, White), lies near 60°. Wind forcing by the prevailing winds is expected to be alongshore in these areas. Two recent studies have confirmed this expectation. In Trinity Bay alongshore wind stress forced alongshore currents which in turn drove a system of upwelling (Yao 1986). Yao reported that the highest coherence between wind and current occurred at periods of 3–7 d and with alongshore winds of 60° true, precisely the periods of maximum coherence and average alongshore orientation of winds on the north shore (Rose and Leggett 1988b). Coherence between alongshore winds and sea temperature variations was significant at periods between 4 and 6 d along the Atlantic coast of the Avalon Peninsula (Schneider and Methven 1988). We conclude that the resolution of the direction of wind forcing in relation to nearshore hydrographics is scale dependent and will result in many site-specific variations, especially on a highly featured coastline such as that found in eastern Newfoundland. In general, alongshore forcing will apply at larger scales (>2 d, coastal indentations wider than the deformation radius) and cross-shore forcing at smaller scales (Csanady 1982; Rose and Leggett 1988b).

Temporal variations in the factors that regulate nearshore currents and sea temperatures, at scales larger than those measured in this study, are also expected. For example, flows through the Strait of Belle Isle are known to vary considerably as a result of larger scale pressure differences between the Labrador Sea and continental eastern Canada (Garrett and Toulany 1981). Rose and Leggett (1988b) hypothesized that variability in flows through the Strait would greatly affect coastal hydrodynamics on the north shore. We believe that variations in the coastal hydrodynamic system at scales other than those studied here will have effects on cod movements and catch in the nearshore zone at corresponding scales.

In conclusion, we have demonstrated that cod movements and catch in the nearshore zone are predictably associated with wind-forced coastal water mass advections, as theorized by Templeman (1966). We have shown the forcing of these advections to be alongshore, however, and not cross-shore as predicted by Templeman, although his predictions of cross-shore forcing are expected to hold at local sites and at small scales which were not resolved in this study. Favorable currents and nearshore water temperatures (-0.5–8.5°C) were a precondition for high catch rates. Favorable physical conditions coupled with high cod abundance in the offshore zone ensured high catch rates at the scales we measured.

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An Empirical Evaluation of the Duration-in-Beam Technique for Hydroacoustic Estimation[1]

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Thorne, R. E. 1988. An empirical evaluation of the duration-in-beam technique for hydroacoustic estimation. Can. J. Fish. Aquat. Sci. 45: 1244–1248.

The duration-in-beam is an empirical technique for estimation of the effective sampling angle of a hydroacoustic beam. The technique potentially allows estimation of fish densities without the usual requirements for system calibration and target strength information. Duration-in-beam measurements from 50 hydroacoustic surveys of juvenile sockeye salmon (*Oncorhynchus nerka*) in Alaska were used to examine the variability in the technique. The results showed reasonable consistency in the beam angle measurement and indicated that the technique was not subject to the estimation errors usually associated with uncertainty in the acoustic system calibration and target strength parameters.

La durée sous le faisceau est une technique empirique permettant d'estimer l'angle d'échantillonnage effectif d'un faisceau hydroacoustique. Cette technique offre la possibilité d'estimer la densité de poisson sans qu'il soit nécessaire d'étalonner le système et d'obtenir des données sur l'indice de réflexion de la cible. Des mesures de durée sous le faisceau obtenues au cours de 50 inventaires hydroacoustiques de juvéniles de saumons nerkas (*Oncorhynchus nerka*) de l'Alaska ont été utilisées pour étudier la variabilité de la technique. Les résultats obtenus indiquaient une bonne cohérence des mesures de l'angle du faisceau et montraient que la technique n'était pas sujette aux erreurs d'estimation généralement liées aux incertitudes de l'étalonnage du système acoustique et aux paramètres de l'indice de réflexion.

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One of the problems with hydroacoustic techniques for estimation of fish populations is the need to determine the effective sampling angle of the hydroacoustic beam. Although the hydroacoustic beam is often depicted as a simple cone with a specified angle, the beam is actually complex (Thorne 1983a; Johannesson and Mitson 1983). The effective angle is a function of several parameters: the acoustic target strength of the fish, the threshold level for detection, the source level and receiver gain of the acoustic system, and the characteristics of the transducer beam, which are described by its directivity pattern (Fig. 1). The manufacturer's specified beam angle is normally measured at the point where the directivity pattern is 3 dB down from its value on the acoustic axis (about 12° in Fig. 1). Usually the effective sampling angle is larger. Theoretically, this angle can be estimated if the above parameters are known. However, the acoustic system parameters, including the directivity pattern, have to be measured in special calibration facilities that are not readily available, and lack of information on target strengths of fish is one of the major current limitations to acoustic assessment (Thorne 1983b; Ehrenberg 1983; Traynor et al. 1987). An alternative approach for estimation of the effective sampling volume of the acoustic beam that requires minimal information is an empirical technique called duration-in-beam. Basically, the effective diameter of the acoustic beam is determined from the average number of times successive echoes are received from individual fish as the transducer moves over the fish at a known speed (any movement of the fish is assumed to be minor compared with the boat speed). This technique has been extensively applied, especially in freshwater environments (Thorne and Thomas 1984), but has lacked thorough evaluation. The objective of this paper is to examine some of the historical duration-in-beam measurements and to investigate characteristics of the variability associated with these measurements and its possible effect on population estimation.

Duration-in-Beam Technique

For the purpose of explaining the technique, assume that the usual situation is reversed, and the transducer is stationary while a fish swims at a known speed directly along the diameter of the cross-section of a continuously sampling acoustic beam (Fig. 2). The diameter of the beam at that depth could be determined by the relationship

$$(1) \quad d = st$$

where s is the swimming speed of the fish and t is the time to swim through the beam.

The time can also be approximated by

$$(2) \quad t = ep$$

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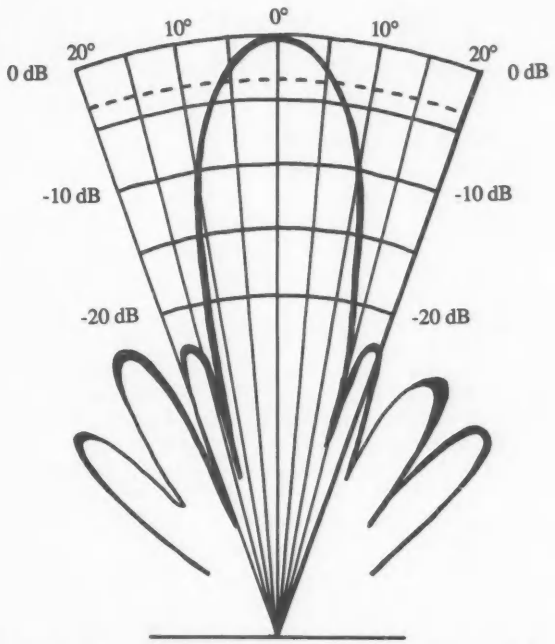

FIG. 1. One-way directivity pattern of Simrad EY-M transducer showing the change in sensitivity (in decibels) with angle from the acoustic axis (0°). The nominal beam width of 12° full angle is denoted by the intersection of the directivity pattern with the −3dB level (broken line).

where e is the number of successive echoes on the fish and p is the pulse repetition rate (number of soundings per unit time).

The probability of a fish swimming directly across the diameter of the acoustic beam is small. However, for straight line swimming paths and a circular transducer pattern, the average path length across the acoustic beam can be determined from simple geometry.

If $\bar{l}$ equals the average path length, than $\bar{l}d$ is the area of a rectangle with equivalent area to the cross-section of a circular beam pattern with diameter d. Then

(3) $\bar{l}d = \pi r^2 = \pi d^2/4$

and

(4) $\bar{l} = \pi d/4$

or

(5) $d = 4\bar{l}/\pi$.

As in equation 2, $\bar{l}$ can be determined from the echoes and pulse repetition rate:

(6) $\bar{l} = s\bar{t} = s\bar{e}p$.

Now reverse the relationship between the fish and the transducer. Assume the usual case where the fish is stationary and the transducer is moving over the fish. In this case, the diameter of a circular acoustic beam can be determined as follows:

(7) $d = 4\bar{e}s/\pi$

where s is now the speed of the boat. The sampling angle can now be estimated by simple trigonometry from the beam diameter and the depth.

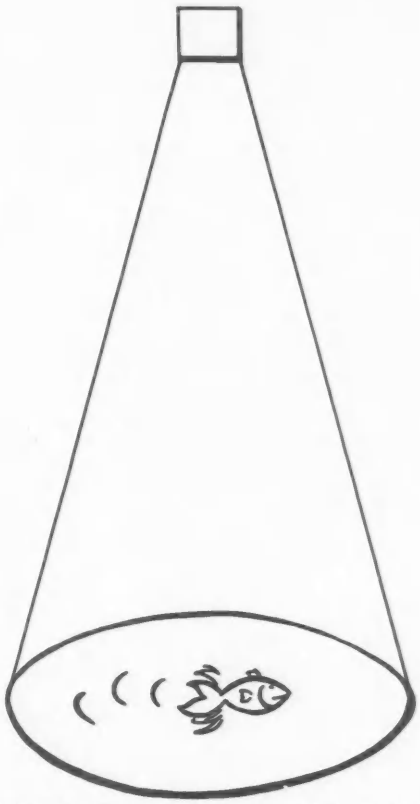

FIG. 2. Illustration of acoustic beam geometry for the duration-in-beam measurement.

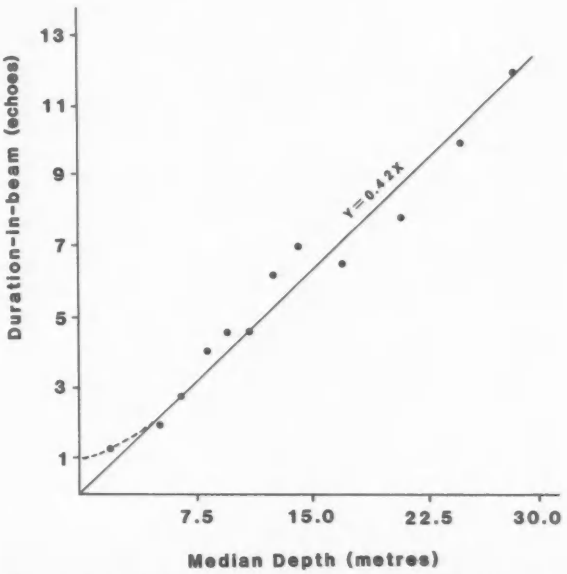

FIG. 3. Duration-in-beam versus depth for Lake McDonald, September 1983, where duration-in-beam is the average number of successive echoes from individual fish at each depth.

This method of estimating the sampling angle from successive echoes (duration-in-beam) was first conceived by R. Thorne and H. Lahore in 1970 and first applied to estimation of juvenile sockeye in Lake Washington in March 1971 (Thorne 1972; Thorne et al. 1973, 1975). It was subsequently used by several investigators at the University of Washington for estimation of populations of sockeye salmon (*Oncorhynchus nerka*) (Croker 1972; Dawson 1972; Nunnallee 1973, 1980).

Duration-in-beam measurements are generally taken from the same data as the population estimate, i.e. from data taken along transects. Usually about 100 duration-in-beam measurements are taken at each of several representative depths, and the mean value at each depth is plotted against depth (Fig. 3).

For any given beam angle, the diameter of the acoustic beam cross-section increases linearly with depth, i.e.

$$(8) \quad d = br$$

where r is the depth or range.

The duration-in-beam values (successive echoes) also follow this model except at lower values. Since 1 is the minimum number of echoes that can be observed, the lower duration-in-beam values converge on a value of 1.0. The bias associated with low duration-in-beam values was examined by Nunnallee (1980). It becomes significant at values below 3 successive echoes.

The sampling angle of the beam is usually estimated by examining the data and regressing depth against the higher (>3) duration-in-beam values, although theoretically, measurement at a single, representative depth is adequate. Crittenden et al. (1987) proposes more quantitative, minimum variance methods for this determination.

Advantages of the Technique

Although the sampling angle is a function of the target strength of the fish, Thorne (1983a) pointed out that echo counting techniques, unlike echo integration techniques, are relatively insensitive to errors in the target strength assumption because of the nature of the directivity pattern. The directivity pattern of a typical circular transducer such as the one used in this study (Fig. 1) has 15–20 dB between the acoustic axis and the side lobes. This corresponds to a 30–40 dB usable dynamic range, since the directivity effects both the transmitted signal and the received signal. When system gains are properly established to match the dynamic gain of the echosounder to the amplitudes of the fish targets (i.e. no fish echoes saturate the system and the smallest fish are detectable on the acoustic axis), the average fish target tends to be detectable at an angle about half way between the acoustic axis and the side lobes, which corresponds to the steep slope of the directivity pattern where the angle changes relatively slowly with large gain or target strength changes. For example, if a fish with a -50 dB target strength were detectable at the -10 dB point on the one-way directivity pattern shown in Fig. 1 (i.e. where its amplitude is -20 dB lower than on the acoustic axis), the effective angle would be 19.5°. Then, a larger target with a -44 dB target strength would be detectable at 21.4° (-13 dB on the one-way directivity pattern), a 10% change. Correspondingly, if the assumed target strength was -50 dB and the real target strength was -44 dB, the estimate would be in error by 10%. The same error in the target strength assumption would result in a 300% error in echo integration analysis.

Since the duration-in-beam technique provides an estimate of the effective sampling angle, that estimate should also be relatively insensitive to variations in the target size, and various surveys of fish stocks with similar size characteristics and similar equipment should produce consistent duration-in-beam measurements.

Evaluation of the Technique

Alaska Lake Survey Data

The duration-in-beam technique has been used extensively in the estimation of sockeye salmon populations in Alaskan nursery lakes since 1982, including the use of consistent equipment and procedures since 1983. Investigations in both southeastern and central Alaska have used Simrad EY-M echosounders with narrow beam (12° nominal beam width) transducers. Survey procedures in the two areas are identical except that boat speed is determined by time and distance in southeastern and by flowmeter in central Alaska. The data base, consisting of 50 hydroacoustic surveys of juvenile sockeye salmon in 14 different lakes over 3 yr, allows evaluation of the variability in the duration-in-beam measurement and an estimate of the amount of uncertainty in the population estimates associated with the method.

Estimated Sampling Angles

The 33 surveys from southeastern Alaska had a mean angle of 18.9° with a standard deviation of 2.02 and a range of 14.6 to 24 (Table 1). The 17 surveys from central Alaska had a mean of 18.6° with a standard deviation of 1.89 and a range of 17–23. One difference between the two areas is the lack of direct speed measurement and control in southeastern Alaska. Errors in the indirect measurement of boat speed directly affect the duration-in-beam estimate and may explain the greater variation in that area. However, regression of estimated angle and boat speed showed no obvious relationship ($r^2 = 0.30$).

The variation in the estimated sampling angles for the various surveys does not necessarily imply error in the measurements. Some of the observed variation in the duration-in-beam measurements undoubtedly represents real change in the sampling characteristics. As explained above, the gain of the echosounding system and the subsequent echo counting threshold are established following general guidelines for matching the dynamic range of the acoustic system to the range of amplitudes of the fish targets. No calibration or target strength information is used in the procedure. Although all 50 lake surveys targeted on sockeye salmon, there was variability in both the size and species composition. Further, noise characteristics of the data affect the choice of the counting threshold. This in turn is influenced by factors such as variation in the depths of fish, wind and wave conditions, and electrical interference. The impact of such factors on the duration-in-beam measurement can be illustrated by the example from the Salmon Lake survey of September 1983 which resulted in the largest measured angle, 24°. Notes recorded during the analysis commented on excellent signal-to-noise characteristics as a result of large targets distributed near surface and perfect weather conditions. The effect of increasing noise level with depth (a result of the time varied gain of the echo sounder) can be seen in the April 1986 surveys of Hugh Smith and Badger Lake. In these cases, signal-to-noise characteristics led to the use of different threshold for shallow versus deep intervals. In both cases the resulting angle from the duration-in-beam was 19° for the shallow depth and 16° for the deeper. Thus, the standard deviation of all measurements is less

TABLE 1. Sampling angles determined from duration-in-beam measurements during Alaskan lake surveys, 1983–86.

Location	Lake	Date (mo/yr)	Angle (°)	Boat speed (m/s)	Comment
Southeastern Alaska	Hugh–Smith	3/83	16	2.00	
		7/83	20	2.99	
		9/83	21	3.08	
		11/83	21	2.55	
		4/84	19.4	2.67	
		7/84	17.6	2.89	
		4/85	19	2.50	
		7/85	20	2.27	
		9/85	20	2.11	
		4/86	19	2.29	16° lower depths
	McDonald	3/83	19	1.90	
		7/83	20	1.90	
		9/83	21	2.98	
		11/83	21	2.62	
		3/84	14.6	2.57	
		7/84	21.4	3.05	
		9/84	18	2.40	
		4/85	22	3.10	
		7/85	18	1.70	
		9/85	19	2.40	
		3/86	16	1.81	
	Falls	10/83	18.4	2.58	
		11/84	19.8	2.02	
		9/85	17	2.60	
	Badger	6/85	16	2.51	
		7/85	19	2.31	
		8/85	17	1.72	
		10/85	17	2.20	
		4/86	19	2.24	16° lower depths
	Redoubt	9/85	17	1.70	
		4/86	17	1.85	
	Salmon	9/83	24	2.40	Large targets,
		9/84	18.6	2.40	clean signal
Central Alaska	Leisure	6/86	17.3	1.5	
		10/84	17.0	1.5	
		8/85	23	1.5	
		10/85	21	1.5	
	Packers	8/85	18	1.5	
		10/85	19	1.5	
	Tokan	8/83	17	1.5	
		8/85	17	1.5	
	Karluk	5/85	18.6	1.5	
		8/85	17	1.5	
		9/85	20	1.5	
	Bear	7/85	22	1.5	
		9/85	18	1.5	
		10/85	19	1.5	
	Larson	7/85	17	1.5	
	Shell	9/85	19	1.5	
	Frazer	8/85	17	1.5	

than the known differences obtained by minor adjustments for different noise levels.

Error in Population Estimation

The variability in the duration-in-beam measurements for the 50 surveys is small and indicates high precision in the estimation procedures. The effect of error on the estimated sampling area of the beam is proportional to the error squared because the sampling area of the beam is proportional to the square of the beam diameter. However, since the number of echoes per target is directly measured in the estimation procedure, the effective error should be linear, i.e. proportional to the

"sweep" of the beam along the transect. In this case, the standard deviation of the 50 duration-in-beam observations would represent about a 10% error in the population estimates if the entire variation was due to error in the measurement. Again, this is unlikely considering the real variability in target amplitudes, thresholds, and system gains, and therefore the error in the population estimates should be less than 10%.

Direct groundtruth comparisons of the resulting population estimates are difficult to obtain, since no other technique can provide reasonably accurate estimates of these populations. The closest measure is annual smolt counts, conducted at most of these lakes, using a variety of traps and weirs. Results have compared well with the hydroacoustic population estimates (G. Kyle, Alaska Department of Fish and Game, Soldotna, AK, and L. Peltz, Alaska ADFG, Ketchikan, AK, pers. comm., and various ADFG internal reports). Serious consideration has been given to eliminating the annual smolt counts in several systems because of their high cost compared with the apparent accuracy and low cost of the hydroacoustic assessments.

Conclusions

The evidence from the Alaskan lake surveys strongly indicates that the duration-in-beam technique provides an accurate and consistent measure of the effective sampling angle of hydroacoustic beams. The technique thus provides a direct, empirical means for absolute population estimation which bypasses the necessity for either rigorous system calibration or detailed knowledge of the scattering characteristics of the fish targets.

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A Weighted Duration-in-Beam Estimator for the Volume Sampled by a Quantitative Echo Sounder

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Estimation of absolute fish density or abundance using the echo-count technique requires estimation of the volume sampled. The unknown parameter in the volume sampled is the sine of the half-angle of the effective conical beam. We derive the minimum variance, unbiased, linear estimator of this parameter. This estimator is based upon weighted regression through the origin and reduces to a computationally simple form. We use this estimate and its variance to estimate absolute density, absolute abundance, and their variances. We use a bootstrap procedure to contrast the performance of our method against that of a contemporary method, which is based upon the averages taken over range strata. Although both of these methods are unbiased, we show that our estimator has a standard deviation that is 23.5% smaller than that of the average-over-strata method.

L'estimation de la densité absolue ou de l'abondance des poissons au moyen de la technique de dénombrement par écho nécessite une estimation du volume échantillonné. Le paramètre inconnu dans le volume échantillonné est le sinus de la moitié d'angle du faisceau conique effectif. Nous en tirons la variance minimale, non biaisée, estimateur linéaire de ce paramètre. Cet estimateur est basé sur la régression pondérée passant par l'origine, et se réduit à une formule simple à calculer. Nous utilisons cette estimation et sa variance pour estimer la densité absolue, l'abondance absolue et leurs variances. Nous nous servons d'une méthode « bootstrap » pour évaluer l'efficacité de notre méthode par rapport à celle d'une méthode contemporaine, qui est basée sur les moyennes prises sur des strates de portée. Bien que ces deux méthodes soient sans biais, nous montrons que notre estimateur a un écart type qui est de 23,5 % inférieur à celui de la méthode des moyennes calculées sur strates.

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In the duration-in-beam approach, the average number of detections per target is used to estimate the effective volume sampled. Target counts can be converted to fish densities by using this volume estimate. These volume and density estimates have often been computed independently for each of several range strata.

Croker (1972), Dawson (1972), Forbes and Nakken (1972), Thorne (1972), Nunnallee (1973), and Thorne et al. (1973, 1975) presented the first applications of the duration-in-beam approach. These first applications used a variety of methods of computation. Nunnallee (1973) described a standard procedure, Nunnallee (1980) examined the sources of error in this method using Monte Carlo simulation, and Nunnallee and Mathisen (1982) presented the derivation of that duration-in-beam estimator. Thorne (1988) examined the performance of this duration-in-beam method in fifty surveys and found it to be both consistent and precise.

Aksland (1985) presented a derivation of the probability function of the number of detections and its first and second moments. Although he treated the number of detections as a discrete variable, rather than as continuous, his results concerning the first moment are consistent with the duration-in-beam literature. However, the second moments differ because of the pulse lag variable he introduced. Aksland's equations are exact, but they are unwieldy. Fortunately, as Nunnallee (1980) showed, error due to the lag between successive pulses is negligible and the number of detections can be treated as a continuous variable when samples are taken using high pulse repetition rates and slow boat speeds. The assumption that the number of detections is a continuous variable allows the development of equations that yield a practical estimation procedure.

We treat the number of detections as a continuous variable and model it as a function of range. We model the volume sampled as a cone, the effective beam. This is illustrated in Fig. 1. Using this model, we derive an estimation procedure to determine the mean and variance of the sine of the half-angle of the effective beam, and we show how to use these values in estimating absolute density and absolute abundance. We also

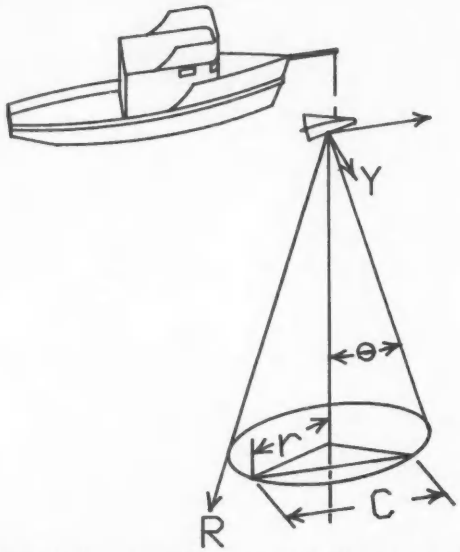

FIG. 1. Effective beam for mobile, downward looking echo sounding, with the coordinates and a chord.

show that this procedure is an improvement over contemporary methods, for although Nunnallee and Mathisen's method is unbiased, it is not minimum variance. By using both the expectation of the number of detections and its variance, we obtain a more precise unbiased estimator which uses the same data.

Recent developments that are especially relevant to the calibration of echo-count data were presented at the 1987 International Symposium on Fisheries Acoustics. Crittenden and Thomas (1987) extended our model to correct the bias due to fish swimming speed, Furusawa and Miyanohana (1987) examined the properties of trace length statistics, and Kieser and Ehrenberg (1987) presented an alternative method of calibration.

Methods

In examining the problem of estimating $\Phi = \mathrm{Sin}(\Theta)$, where Θ is the half-angle of the effective beam, we use the following simplifying assumptions: (1) the directivity pattern is a monotonically decreasing function of the angle off-axis (θ) and depends upon no other variable describing the spatial orientation; (2) fish have equal target strengths; (3) fish are isolated single targets which can be distinguished from the background noise; (4) fish movement is negligible relative to the boat's speed; (5) at any range, R, the fish are independently uniformly distributed across the effective beam, perpendicular to the boat's direction of motion, that is $[f_y(y) = 1/(2r), (-r \leq y \leq r)]$, where $r = R\Theta$, and R is one half the distance from the center of the transducer's face to the fish at its first detection plus one half that distance at its last detection. Notation is summarized in Table 1.

The first step in the derivation is to obtain the probability density function (pdf) of the cord length, from the pdf of y. The cord length (C) along which a fish passes through the effective beam is obtained using the Pythagorean theorem ($C^2/4 = r^2 - y^2$). The change of variables formula (Hoel et al. 1971) is used to obtain the pdf of the cord lengths:

TABLE 1. Notation.

A	surface area of the waterbody surveyed (m^2)
B	number of bootstrap runs
C	chord length (m)
f	probability density function
H	dummy variable
i	index for the individual observations
j	index for the strata
K	$(\Phi^2 \cos(\arcsin(\Phi)))^{-1}$
M	number of pulses per sequence
m	number of depth strata
N	abundance of the fish population
n	sample size
p	pulse repetition rate (s^{-1})
r	radius of the effective beam (m)
S	the boat's speed (m/s)
V	effective volume sampled (m^3)
W	statistical weight
y	distance perpendicular to the boat's course (m)
R	range (m)
β	regression coefficient
ϵ	random error
η	duration-in-beam, zeros omitted
Θ	half-angle of the effective beam (radians, dimensionless)
θ	angle from the acoustic axis (radians, dimensionless)
κ	correction factor for the surface layer
λ	probability that ($\nu \neq 0$)
ν	number of detections
ρ	fish population density ($fish/m^3$)
ρ_a	fish population areal density ($fish/m^2$)
Φ	$\sin(\Theta)$
$\hat{z}$	circumflex denotes the estimate (e.g. of z)
$\bar{z}$	macron denotes the arithmetic mean (e.g. of z)
$\tilde{z}$	tilda denotes a relative value (e.g. of z)

$$(1)\quad f_C(C) = f_y(y)\,|\,dy/dC\,| = (C/4r)\,(r^2 - C^2/4)^{-1/2} \qquad (0 \leq C \leq 2r)$$
$$= 0 \qquad (C < 0 \text{ or } 2r < C).$$

In making this calculation, the pdf must be derived separately for ($y < 0$) and ($y \geq 0$) and then combined. This is necessary to meet an assumption underlying the change of variables formula, that the back-transformation, from C to y, is one-to-one.

In order to obtain the pdf of the number of detections, we introduce the approximation that ($\nu = Cp/S$), where ν is the number of detections from a fish, p is the pulse repetition rate, and S is the boat's speed. In this approximation, we treat the number of detections as a continuous variable.

The change of variables formula is then used to obtain the pdf of ν:

$$(2)\quad f_\nu(\nu) = f_C(C)\,|\,dC/d\nu\,| = \frac{\nu S^2}{4rp^2\left(r^2 - \dfrac{\nu^2 S^2}{4p^2}\right)^{1/2}} \qquad (0 \leq \nu \leq 2rp/S)$$
$$= 0 \qquad (\nu < 0 \text{ or } 2rp/S < \nu).$$

We obtain the expectation of ν by using the definition of expectation:

$$(3)\quad E(\nu) = \int_0^{2rp/S} \nu f_\nu(\nu)\,d\nu = \frac{\Phi\pi p}{2S} R.$$

Substituting the sample mean for the expectation and rearrang-

ing gives Nunnallee and Mathisen's estimator. They computed their estimator for each range stratum, j, and then averaged it over the m strata, using the equation

$$(4)\quad \hat{\Phi}_{NM} = \sum_{j=1}^{m} (\bar{\nu}_j 2S/(R_j \pi p))/m.$$

Taking the expectation of this equation shows that Nunnallee and Mathisen's estimator is unbiased.

Equation (3) can be expressed, for the ith observed fish, in the form of a model that is linear in R, has a slope ($\beta = \Phi\pi p/2S$), and passes through the origin:

$$(5)\quad \nu_i = \beta R_i + \epsilon_i$$

where

$$\epsilon_i = \nu_i - E(\nu_i).$$

In this model, the errors are heteroscedastic because the variance of ν is a function of the range. We compute the variance of ν by using the definition of variance

$$(6)\quad \mathrm{Var}(\nu) = \int_0^{2rp/S} (\nu - E\nu)^2 f_\nu(\nu)\, d\nu$$

$$= \frac{p^2\Phi^2}{S^2}\,(8/3 - \pi^2/4)\,R^2.$$

Draper and Smith (1966) and Seber (1977) showed that linear regression based upon a model which is correct but in which the errors are heteroscedastic gives an unbiased estimate that is not minimum variance. They also showed that the minimum variance, unbiased, linear estimate (BLUE for best linear unbiased estimate, a synomym) is obtained by weighted regression where the weights are proportional to the reciprocal of the square root of the known variances. Therefore we suggest the use of weights of ($W_i = 1/R_i$) for this application.

The estimated slope of this weighted regression and its variance can be computed using the equations

$$(7)\quad \hat{\beta}_{BLUE} = \frac{\Sigma(W_i \nu_i R_i)}{\Sigma W_i R_i^2} = \frac{\bar{\nu}}{\bar{R}}$$

$$(8)\quad \mathrm{Var}(\hat{\beta}_{BLUE}) = \frac{\Sigma(W_i \nu_i) - (\Sigma W_i \nu_i R_i^2)}{(n-1)\,(\Sigma W_i R_i^2)}$$

where n is the number of targets observed.

The minimum variance, unbiased, linear estimate of ($\Phi = \sin(\Theta)$) is obtained from the estimated slope using the linear transformation

$$(9)\quad \hat{\Phi}_{BLUE} = 2S\hat{\beta}_{BLUE}/(\pi p).$$

This is the minimum variance, unbiased, linear estimator, but it differs from Nunnallee and Mathison's estimator. As their estimator is linear and unbiased, theirs must not be minimum variance.

The variance of our estimate can be calculated using the equation

$$(10)\quad \mathrm{Var}(\hat{\Phi}_{BLUE}) = (2S/(\pi p))^2 \mathrm{Var}(\hat{\beta}_{BLUE}).$$

Bootstrap

Efron's (1979, 1982) bootstrap is used in several applications in this paper. Efron and Tibshirani (1986) presented a review of the basic ideas. The bootstrap is a Monte Carlo method in which data sets are simulated by sampling (with replacement)

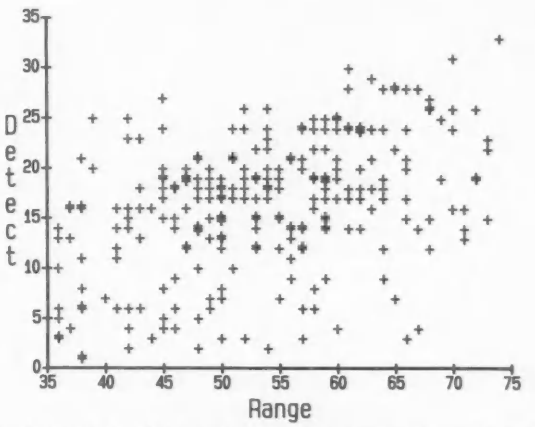

FIG. 2. Scatterplot of the raw data from Crater Lake. The numbers of detections are plotted against the range, with (n = 250).

from the observed vector of random errors. These observed errors must be independent and identically distributed. In this application, because the model is linear and heteroscedastic, the trend and weighting must be taken into account in order to make the observed errors identically distributed. This method has six steps: (1) the weighted regression in our method is applied to the original data set to yield the predicted durations ($\hat{\nu}_i$); (2) compute a vector of independent and identically distributed weighted residuals by using the equation [$\bar{\epsilon}_i = (\nu_i - \hat{\nu}_i)/R_i$]; (3) randomly sample n cases (with replacement) from the vectors ($\hat{\nu}$, R) to create a new pair of vectors ($\hat{\nu}^*$, R^*), independently sample n cases (with replacement) from the weighted residuals ($\bar{\epsilon}$) to create a new vector ($\bar{\epsilon}^*$), and then combine these vectors to create a new data set of sample size n by using the equation [$(\nu^*, R^*) = (\hat{\nu}^* + R^*\bar{\epsilon}^*, R^*)$]; (4) compute any function Q (ν^*, R^*); (5) repeat steps 3 and 4 B times and accumulate the distribution of Q; (6) any statistic of the accumulated distribution can then be estimated. In particular the bootstrap estimate, bootstrap variance, and bootstrap probability are calculated using the equations

$$\hat{Q}_{BS} = \Sigma_{i=1}^{B} Q_i/B$$

$$\mathrm{Var}(\hat{Q})_{BS} = \Sigma_{i=1}^{B} (Q_i - \hat{Q}_{BS})^2/(B-1)$$

$$\mathrm{Prob(event)} = \text{(number of occurances)}/B.$$

Data

We use data taken during August 1985 at Crater Lake, Oregon, to examine the performance of our estimator. Marino (1987) described the survey. It consisted of nine transects. Three of these transects went across the entire lake and the other six transects followed zigzag courses between the shore and the 91.5-m contour.

The equipment we used for this survey was a dual-beam echo sounding system. This consists of a Biosonics 101 dual-beam receiver, a 420-kHz transceiver, a Ross 250C chart recorder, a Biosonics 171 recording interface, a digital signal processor, a beta-format video cassette recorder, and a 6 degree/15 degree dual-beam transducer (where these are the angles between the acoustic axes and the half-power isopleths). We used a portable dual-trace oscilloscope to monitor system performance. The transducer was housed in a v-fin which we suspended from the boat at a depth of 0.5 m.

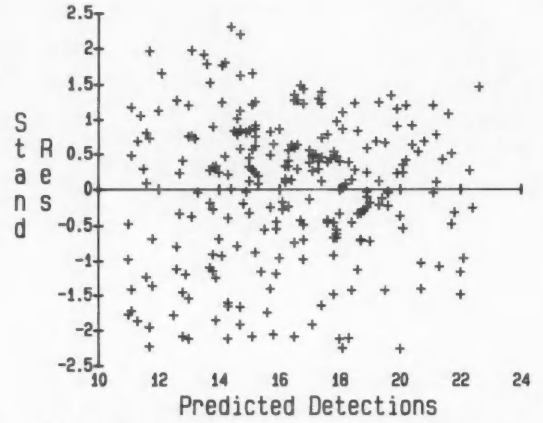

FIG. 3. Standardized residuals plotted against the predicted number of detections (n = 250).

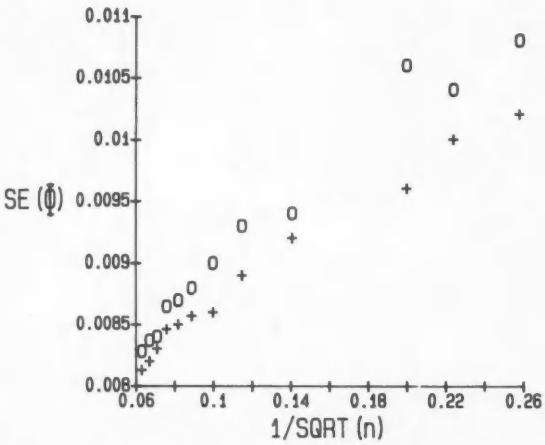

FIG. 4. Bootstrap standard deviations of $\hat{\Phi}$ plotted against the reciprocal of the square root of the sample size for our method (crosses) and the average-over-range method (circles).

The selection of system settings was based on the results of the survey by Thomas et al. (1985) using the same calibrated system where the smallest fish size was an age 0 sockeye salmon (*Oncorhynchus nerka*). We used a 40 log range time varied gain. The pulse repetition rate was (p = 4/s), the pulse width was 0.5 ms, and the boat's speed was (S = 1.69 m/s). Recorder gains were set so that the largest signals were just below saturation, as suggested by Bruczinski (1984).

We played back the tape recorded data using the same equipment described above and counted echoes using a dual-beam storage oscilloscope. Fish population densities were low enough that the fish appeared as isolated targets. This allowed processing using the echo-counting method, as suggested by Thorne (1983) and Nunnallee (1973). We observed 733 targets and measured them individually. We measured the variables time of day, location, range, number of detections, and peak voltage for the narrow beam. In order to obtain data from only one size class of fish, only data from ranges between 35 and 75 m are used. The raw data are shown in Fig. 2. There are [n = 253] observations. We omitted three aberrant observations for reasons discussed below.

Results

Using our method, the estimated model is $v = 0.30855\,R$, $\text{SE}(\hat{\beta}_{\text{BLUE}}) = 0.007913$. The standardized residual plot is shown in Fig. 3. It is homoscedastic without curvilinearity. From this regression we obtain an estimated value of the sine of the half-beam angle of $\hat{\Phi}_{\text{BLUE}} = 0.08299$, $\text{SE}(\hat{\Phi}_{\text{BLUE}}) = 0.00197$.

We used the bootstrap to compare the two estimators, $\hat{\Phi}_{\text{BLUE}}$ and $\hat{\Phi}_{\text{NM}}$. We used 10-m depth strata in computing $\hat{\Phi}_{\text{NM}}$. Samples sizes (n) of 15–250 were examined and [B = 50] runs were done at each sample size. The bootstrap standard errors are shown in Fig. 4 plotted upon the reciprocal of the square root of the sample sizes. The regression coefficients are significant (at the α = 0.01 level) and the regression equation is $[\text{SE}(\hat{\Phi})_{\text{BS}} = (0.0318 + 0.00976H)/\text{SQRT}(n)]$. H is a dummy variable for the method of analysis (H = 0 for $\hat{\Phi}_{\text{BLUE}}$ and H = 1 for $\hat{\Phi}_{\text{NM}}$). This regression is a paired comparison analysis of covariance.

Discussion

These simulation results show that for the data set examined, the standard error of $\hat{\Phi}_{\text{NM}}$ is 30.7% larger than the standard error of $\hat{\Phi}_{\text{BLUE}}$. From the derivation of $\hat{\Phi}_{\text{BLUE}}$, we know that our method should improve the precision of the estimate. The simulation shows that this improvement can be substantial.

Nunnallee and Mathisen's estimator is usually computed using the mean range of each stratum. In contrast, for our method, the range of each observation is measured as exactly as possible and is treated explicitly. However, when all the observations are at the same range, these two estimators are identical. The weighted method has an advantage over Nunnallee and Mathisen's method only when the observations are taken at a variety of ranges. However, this is the usual situation.

Nunnallee and Mathisen's estimator could be computed using the observed range for each fish target. Using this approach, their estimator reduces to a constant times the mean of the ratio (v_i/R_i). In contrast, our method is the same constant times the ratio of the means ($\bar{v}/\bar{R}$). Cochran (1977) discussed the properties of these alternative estimators, the mean ratio versus the ratio of the means. His conclusions are consistent with ours.

Although equations (7) and (8) are presented to simplify computation by hand, we suggest that analysis be done as a weighted regression through the origin, using a computer statistical package. This approach is heuristic and computer packages can allow the easy production of scatter and residual plots. These plots are invaluable to examination for departures from the assumptions. The standardized residuals should be plotted against the predicted number of detections and also against the ranges. These plots should be examined for heteroscedasticity, outliers, and trends. Weisberg (1980) presented a readable review of modern regression procedures.

Detections of Zero

A bias arises in the sampling because fish can pass through the volume sampled between transmissions, without being detected [v = 0]. In both our approach and Aksland's (1985), these zeros are predicted to occur; but they are not measured and do not appear in the data set. Their absence causes bias. Specifically, it causes the average number of detections to be larger than expected. This effect is usually negligible except where the expected number of detections is small. Thorne (1988) presents several figures showing this effect. This effect

is not noticeable in his figures for expected numbers of detections greater than 3.

This bias due to neglecting the zeros can be estimated based on a model for the number of detections conditional upon observed values being one or greater. Zeros are treated as discrete events with probability

$$(11)\quad 1-\lambda = \text{Prob}(\nu = 0) = \int_0^1 (1-\nu)\, f_\nu(\nu)\, d\nu$$

$$= \frac{\left(r^2 - \frac{S^2}{4p^2}\right)^{1/2}}{2r} + \frac{rp}{S} \arcsin\left(\frac{S}{2rp}\right).$$

The number of detections with zeros omitted (η) is described using a continuous distribution, but with a single discrete point at 1:

$$f_\eta(\eta) = f_\nu(\eta)/\lambda \qquad (1 < \eta \leq 2rp/S)$$

$$(12)\quad \text{Prob}(1) = \left[\frac{rp}{S} \arcsin\left(\frac{S}{2rp}\right) - \frac{1}{2r}\left(r^2 - \frac{S^2}{4p^2}\right)^{1/2}\right]/\lambda$$

$$f_\eta(\eta) = 0 \qquad (\eta < 1 \text{ or } 2rp/S < \eta).$$

The expectation is

$$(13)\quad E(\eta) = E(\nu = \eta)/\lambda.$$

This equation does not lend itself to a simple estimation method for Φ. Consequently, we concur with Nunnallee's (1980) recommendation to use only data from larger ranges, in which few zeros are expected. In order to approximate the size of the bias that would be introduced, the expectation can be computed using equation (13) and a value of $\hat{\Phi}_{\text{BLUE}}$ estimated ignoring the bias.

Aksland (1985) also recognized the difficulties that arise from small numbers of detections. He presented equations for the probability of [$\nu = 0$] and for the expected number of detections with zeros omitted.

Outliers

The Crater Lake data set (35–75 m) contained 3 (of 253) data points with unusually large counts. These counts were just less than $4rp/S$, almost twice the largest possible number of detections under the model. These three observations may represent instances where one fish entered the effective beam just as another was leaving, at the same range. This should be anticipated, as it will happen occasionally. Other possible causes for such aberrant observations are unusually large fish or fish swimming in the boat's direction at a nonnegligible speed. Aberrant data points, like these, should appear as outliers in the residual plots. As these points do not meet the assumptions underlying the method of analysis, these observations should be treated separately and the remaining data should be reanalysed without them.

Systematic Variation in the Half-Beam Angle

Often, fish of different sizes may occupy different depths in the water column. This may result in a curvilinear trend in the residual plots. In the Crater Lake data analysed, smaller fish occupied depths down to 30 m. A curvilinear trend was evident in the residual plots. Examination of the scatterplot of the target strengths against range confirmed the size variation with depth. Consequently, data from the two depth zones were analysed separately.

Although we had target strength data and were able to use it to verify a difference of fish size, such data will often not be available. We therefore present a test for a difference in the beam angle between two depth zones, based solely upon data on the number of detections and the range. The conventional test based on regression is not applicable due to nonnormality. We present a test based on the bootstrap. A dummy variable ($H = 0$ or 1) is introduced to designate which range zone an observation lies in. The dummy variable equals 1 for the zone with the suspected larger beam angle. A regression through the origin is then computed based on the model ($\nu_i = \beta_1 R_i + \beta_2 H_i R_i + \epsilon_i$), with weights ($W_i = 1/R_i$). The bootstrap probability of the event ($\beta_2 < 0$) is computed and compared with the desired alpha level. As Efron and Tibshirani (1986) pointed out, the bootstrap can be computationally intensive for examining probabilities in the tails. For example at [$\alpha = 0.05$], only 25 events would occur in the tail at [$B = 500$] runs.

To avoid problems with a posteriori testing, the test needs to be conducted on a second independent set of data, after an apparent trend was observed in the first data set. This should not pose a difficulty when the data sets used to estimate the half-beam angle are obtained by subsampling a much larger data set. This will often be the sampling design when the objective is to survey fish abundance.

Random Variation in the Half-Beam Angle

Even within a narrow zone of depths, there will almost always be some variation in the fishes' target strengths. This variation may result from differences in the sizes and orientations of the fish. If this variation is random and independent of the range, there is no difficulty: the expectation of the half-angle will be unaffected, and its variance can be computed based on the conditional variance formula (Rao 1973; Kendall and Stuart 1976);

$$(14)\quad \text{Var}(\nu) = \text{Var}_\Phi E(\nu|\Phi) + E_\Phi \text{Var}(\nu|\Phi)$$

$$= \text{Var}_\Phi\left(\frac{\Phi \pi p R}{2S}\right) + E_\Phi\left(\frac{8\Phi^2 p^2 R^2}{3S^2}\right)$$

$$= R^2\left[\frac{\pi^2 p^2}{4S^2}\text{Var}(\Phi) + \frac{8p^2}{3S^2}(Var(\Phi) + E^2\Phi)\right].$$

This result shows that the variance of ν remains proportional to R^2 despite random variation in Θ. Hence, the weighted estimator remains appropriate even when Θ is a random variable. This random variation will be automatically included in the variance of the estimate.

This treatment of the effects of random variation in the half-beam angle shows that we can relax the restriction of constant target strength. As the half-beam angle can vary randomly, independent of the range, so can the target strength, or any other variable which has that same effect. Examples may include variation due to pitching or rolling of the transponder's towed housing, small electronic fluctuations, and variation in the criteria for counting targets.

Maximum Likelihood Estimation

The maximum likelihood estimate (MLE) can be obtained based on the pdf in equation (2). We found that this MLE is sensitive to variation in Θ. This occurs because the MLE must be such that all the observations are possible. This restricts the MLE to values such that

(15) $\hat{\Phi}_{ML} \geq \text{Max} \left(\frac{v_i S}{2 R_i p} \right)$.

This makes the MLE sensitive to outliers. They can be the dominant factor controlling the MLE, and as a result, the MLE can have a substantial positive bias in this application.

Distribution-Free Regression

The distribution-free regression methods suggested by Theil (1950) and Brown and Mood (1951) can be used to obtain quick preliminary estimates. For this application, the regression model is given by equation (5). Since this model is for regression through the origin, Theil's and Brown and Mood's methods need to be slightly modified.

Theil's method uses the median slope of the lines defined by all pairs of data points. For regression through the origin, Theil's estimate is the median slope of the lines defined by the origin and each datum point. This reduces to $[\hat{\beta}_{DF} = \text{Median}\ (v_i / R_i)]$.

Brown and Mood's method is graphical and is done by placing a ruler on the scatterplot of v against R. For regression through the origin, the ruler is placed so it passes through the origin and is then rotated until an equal number of points lie above and below the ruler. If a line is to be fitted by eye, we suggest this method. On the average, this gives the same result as Theil's method.

In order to obtain an unbiased estimate ($\hat{\Phi}_{DF}$) from the above distribution-free slope estimates, we solve for the median of (v/R) using the distribution given in equation (2). This gives the unbiased estimator

(16) $\hat{\Phi}_{DF} = \frac{\hat{\beta}_{DF} S}{3^{1/2} p}$.

Density Estimation

Fish population densities (ρ) have often been estimated by dividing the number of observed echoes (Σv) by the volume sampled (V). The volume sampled in M successive transmissions is computed using the equation for the volume of a conic frustum:

(17) $V = M\pi\ (R_2^3 - R_1^3)/(3K)$

where $[K = 1/(\Phi^2 \cos(\arcsin(\Phi)))]$.

However, if this volume is estimated with error, division of the total counts by the volume sampled gives a biased estimate of the density. This occurs because divisions by V is a nonlinear transformation, and a nonlinear transformation of an unbiased estimate generally yields a biased estimate. In computing observed densities, only K is estimated with error. Hence, we break density estimation into three steps in order to separately address the effects of the nonlinear transformation of the estimated variable: (1) calculate the observed relative density $[\tilde{\rho} = 3\Sigma v/(M\pi(R_1^3 - R_2^3))]$; (2) estimate K and its variance by using the bootstrap; (3) multiply these two terms to obtain the absolute density $[\rho = \tilde{\rho}K]$.

The first step is a computation involving values measured without error. That is, we compute the relative density conditional upon the echo-counts, and assume the error in the range measurements to be negligible.

The second step involves correcting the bias due to nonlinear transformation. We found that the delta method is unreliable for estimating the variance of K. As Seber (1982) pointed out, the bootstrap provides a means of estimating means and variances where awkward transformations make the delta method unreliable. We obtained bootstrap estimates with $[n = 250]$ and $[B = 500]$ runs. These estimates are $\hat{K}_{BS} = 147.62$ and $SE(\hat{K})_{BS} = 6.97$. The bias due to transformation is negligibly small for the Crater Lake data set. Ignoring the bias correction gives ($K \approx 145.69$). Nevertheless, the bootstrap is still needed to compute the variance.

The third step is a linear transformation. The mean and variance can be obtained using the equations $[\hat{\rho} = \tilde{\rho}\hat{K}_{BS}]$ and $[\text{Var}(\hat{\rho}) = \tilde{\rho}^2 \text{Var}(\hat{K})_{BS}]$.

Abundance Estimation

We use Nunnallee's (1973) method for obtaining areal densities and Thorne's (1983) correction for the surface layer. These methods are effective and are currently used. We modify these methods slightly in order to use relative rather than absolute density. This allows us to estimate the variance of the absolute abundance.

The relative densities may be pooled vertically to yield relative areal densities ($\tilde{\rho}_a = \Sigma\tilde{\rho}$). Vertical pooling is outlined by Nunnallee (1973). This pooling condenses the data set and also removes the difficulties due to vertical autocorrelation. This summation should be taken over the entire sampled water column, except the surface layer.

Fish avoidance of the boat can make measurements taken near the surface unreliable. Thorne (1983) introduced a correction for this. The profile obtained from an upward looking stationary survey is contrasted against the profile from the downward looking mobile survey, in order to identify the depth of the surface layer throughout which the profiles differ. A correction factor (κ) is computed from the upward looking stationary survey data. It is the ratio of the pooled density for the entire water column to the pooled density with the surface layer omitted. The variance of κ should also be calculated.

There are many methods for estimating absolute abundance (N) from the data set of observed densities and their spatial locations. These include methods based on replication, clustering, stratification, successive differences, multiple regression, and kriging. Some of these methods use the data set more efficiently than others, but they also differ in their underlying assumptions. Hence, the choice of an appropriate method of analysis should depend upon the circumstances of the individual application. Shotton (1979), Crittenden and Mathisen (1983), and Shotton and Bazigos (1984) reviewed the application of some of these methods to hydroacoustic surveys.

We assume here that one of these methods or an alternative has been chosen. The chosen method needs to yield an estimate and variance of the integral over the survey area (A) of the relative areal density (with the surface layer omitted). The integrated value $[\tilde{N} = \int_A \tilde{\rho}_a dA]$ is the relative abundance. We introduce $[\kappa K]$ to estimate the absolute abundance $[\hat{N} = \tilde{N}\hat{\kappa}\hat{K}_{BS}]$ and its variance $[\text{Var}(\hat{N}) = (\hat{\kappa}\hat{K}_{BS})^2 \text{Var}\ (\tilde{N}) + (\tilde{N}\hat{\kappa})^2 \text{Var}(\hat{K})_{BS} + (\tilde{N}\hat{K}_{BS})^2 \text{Var}(\hat{\kappa})]$. This calculation of absolute abundance is a linear transformation of the three independent variables $\tilde{N}$, $\hat{\kappa}$, and $\hat{K}$.

Advantages of the Duration-in-Beam Method

There are two major alternatives to the duration-in-beam approach. These are echo-integration and echo-counting, calibrated by using what we call the "echo-amplitude" approach.

Echo-counting and the duration-in-beam methods can be used only when the targets are isolated. In contrast, echo-integration can be applied to surveys where the targets are either isolated or not. The precision of echo-counting and echo-integration have been explicitly examined. Ehrenberg (1973) showed that echo-counting is more precise for isolated targets, given known calibration constants for each method.

Bayona (1984) reviewed calibration methods for echo-integration. Several alternative methods are currently used. These are mostly of two approaches: (1) the calibration constants may be estimated by empirical scaling against known or estimated densities and (2) the parameters in question can be estimated directly. In this second approach, an estimate is often obtained of the mean back-scattering cross-section via target strength analysis, and the system characteristics are determined by direct physical measurement. For target strength analysis, the echo voltage level from isolated targets needs to be measured. There are three major approaches taken to target strength analysis. Clay and Heist (1984) showed how to estimate the mean back-scattering cross-section from such data using a single-beam system; Traynor and Ehrenberg (1979) discussed a method using a dual-beam system; and Foote et al. (1986) applied a method using a split-beam system.

The echo-amplitude approach for calibrating echo-count data is reviewed in Forbes and Nakken (1972); and Kieser and Ehrenberg (1987) presented the most recent echo-amplitude method. In the echo-amplitude approach, the same measurements are taken as for direct calibration of echo-integrators. The sonar equation is used to calculate the volume within which detections would be anticipated to occur. In applying the echo-amplitude approach, densities are estimated without the necessity of ever explicitly estimating the effective volume sampled.

Direct calibration of echo-integrators and the echo-amplitude approach both require the measurement of additional variables and the use of additional equipment. This requires additional work and expense. Also, as Craig (1984), Blue (1984), and Robinson (1984) pointed out, error in the measurement of the system characteristics can introduce significant sources of variance or bias.

In contrast, the duration-in-beam estimators do not require the measurement of additional variables, do not require additional equipment, and the measurement errors in the system characteristics do not contribute to the variance, nor do they introduce bias. The only variables that are needed to make a duration-in-beam estimate are the number of detections and the range. These are the variables measured in an echo-counting survey.

The echo-amplitude and duration-in-beam methods differ in their fundamental approaches. In the echo-amplitude methods, all the variables believed to affect the volume sampled are measured, and then an estimate is made by using a theoretical relationship, the sonar equation. In the duration-in-beam methods, a property of the geometry of the volume sampled is measured directly.

These alternative approaches to calibration have different strengths and weaknesses, but have yet to be explicitly contrasted. Their relative accuracy and precision is not known.

In situ measurements need to be employed in calibration. Fish target strengths and system performance affect the half-beam angle, but may vary among and within surveys. For our method, the data set can be the echo-counting survey data set itself. Hence, our method can yield estimates of the values realized under the actual conditions during that survey, and the variance of the estimate will incorporate the variations that occurred during that survey.

The limitations of our duration-in-beam method are explicitly stated in the derivation. The most restrictive of these assumptions are that the targets must be isolated, fish swimming speeds must be negligible relative to the boat's speed, and the expected number of detections must be at least moderate. This sets constraints on the boat's speed, the depths surveyed, the pulse repetition rate, and the beam width. It also limits the surveys to nonschooling fish at low population densities.

The principal uses of the duration-in-beam method have been echo-counting surveys of lakes. These are usually conducted during the night, when many fish schools disperse. As Bayona (1984) pointed out, the duration-in-beam method is also used for the empirical calibration of echo-integrators.

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Immunochemical Quantification of Metallothioneins of a Marine Mollusc

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Roesijadi, G., M. E. Unger, and J. E. Morris. 1988. Immunochemical quantification of metallothioneins of a marine mollusc. Can. J. Fish. Aquat. Sci. 45: 1257–1263.

Mercury-induced, low molecular weight, metal-binding proteins were isolated from the marine mussel *Mytilus edulis* and used as antigen in the development of antibodies and an enzyme-linked immunosorbent assay (ELISA) for quantification of the proteins. Partial characterization of the isolated protein indicated that its properties were consistent with those of metallothionein (MT). In contrast with most MTs, this protein occurred as an apparent dimer and contained glycine at high levels. Polyclonal antibodies against this protein were produced in goats and purified to an IgG fraction by ammonium sulfate precipitation and DEAE ion-exchange chromatography. Ouchterlony analysis and ELISA showed that these antibodies were cross-reactive with two other charge variants of mercury-induced, metal-binding proteins of *M. edulis*, but not with rabbit MT. The ELISA was based on an indirect, competitive procedure utilizing a rabbit anti-goat IgG – horseradish peroxidase conjugate as the second antibody. Application of this assay to cytosolic extracts of mussel gills indicated 0.5 μg metal-binding proteins/g wet tissue weight in gills of control mussels and elevated levels up to 1780 μg/g following exposure to mercury, cadmium, or copper for 28 d. Zinc was not effective as an inducer of these proteins.

Des métalloprotéines de faible poids moléculaire, induites par le mercure, ont été isolées chez la moule commune, *Mytilus edulis*, et utilisées comme antigène pour la production d'anticorps et la mise au point d'un test ELISA pour le dosage des protéines. Une caractérisation partielle de la protéine isolée a montré que ses propriétés correspondaient à celles d'une métallothionéine. Contrairement à la plupart des métallothionéines, cette protéine se présente sous forme d'un dimère apparent et contient une teneur élevée en glycine. Des anticorps polyclonaux dirigés contre cette protéine ont été produits chez des chèvres et purifiés en IgG par précipitation au sulfate d'ammonium et par chromatographie sur colonne échangeuse d'ion (DEAE). La réaction d'Ouchterlony et l'épreuve ELISA ont montré que ces anticorps présentaient une réaction croisée avec deux autres variants de charge des métalloprotéines induites par le mercure de *M. edulis*, mais non avec la métallothionéine de lapin. L'épreuve ELISA est fondée sur une technique de compétition indirecte utilisant un conjugué peroxydase extraite du raifort – IgG anti-chèvre de lapin comme deuxième anticorps. L'application de cette épreuve à des extraits cytolisiques de branchies de moule a montré que les branchies des moules témoins contenaient 0,5 μg de métalloprotéine par gramme de poids humide de tissu et des teneurs pouvant atteindre 1 780 μg/g après une exposition de 28 d au mercure, au cadmium ou au cuivre. Le zinc n'agissait pas comme inducteur de ces protéines.

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Metallothioneins (MTs) are cysteine-rich, low molecular weight, metal-binding proteins whose putative functions include detoxification, storage, and regulation of copper, zinc, cadmium, and mercury (recent reviews by Cousins 1985; Hamer 1986). Studies by Hamer et al. (1985) and Thiele et al. (1986) that have shown that deletion and reinsertion of the MT gene in yeast results in elimination and recovery, respectively, of inducible metal tolerance have strengthened proposals that a primary function of MTs is one of metal detoxification. These proteins have been detected in diverse organisms and are ubiquitously distributed in the animal kingdom, some fungi, and prokaryotes (Hamer 1986). In numerous studies, marine and freshwater fish and invertebrates have been shown capable of synthesizing increased levels of MTs or similar proteins in response to metal exposure (Roesijadi 1981; Klaverkamp et al. 1984). As with other organisms, apparent induction of these proteins in aquatic animals is associated with increased tolerance to the toxicity of the metals identified above (e.g. Roesijadi et al. 1982; Roesijadi and Fellingham 1987; Benson and Birge 1985).

In the marine mussel *Mytilus edulis*, MT-like metal-binding proteins are present in organs such as gills, digestive gland, and kidneys and probably associated with pathways for metal

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uptake, storage, and excretion (Roesijadi 1982; Viarengo et al. 1981; George and Pirie 1980). The application of specific assays for these proteins will be helpful in advancing our understanding of trace metal metabolism in this frequently studied molluscan species.

Immunochemical techniques for the specific and sensitive assay of MTs (Garvey et al. 1982) are based on antibodies against proteins isolated from mammals, which show little cross-reactivity with similar proteins in fish (Kay et al. 1986) or invertebrates (Nordberg et al. 1986). We report here the development of antibodies against a low molecular weight, metal-binding protein isolated from the marine mussel *M. edulis* and antigen detection based on an enzyme-linked immunosorbent assay (ELISA). This protein possessed characteristics consistent with those of MT.

Materials and Methods

Animals

Mussels were collected from Sequim Bay, Washington, and exposed for 28 d to 5μg Hg/L (as $HgCl_2$) under conditions of flowing seawater in the laboratory as previously described (Roesijadi 1982). Since relatively high levels of metal-binding proteins are induced in gills by this treatment, this organ served as the source of metal-binding proteins in this study. Following exposure as described above, the gills were excised and stored at −70°C.

Chemicals

All chemicals were reagent grade or better and were purchased from Baker, Bio-Rad, Eastman, Mallinkrodt, or Sigma chemical companies.

Isolation of Metal-Binding Proteins

Low molecular weight, mercury-binding proteins were isolated from the gills of mercury-exposed mussels, as described by Roesijadi (1986), for later use in antibody and immunoassay development. This procedure basically entailed gel chromatography of the cytosolic fraction of tissues on Sephadex G-75, rechromatography of the main mercury-binding protein peak, which eluted with a molecular mass of about 20 kilodaltons (kDa), on the same medium to remove overlapping higher and lower molecular weight substances, and anion-exchange chromatography of the purified proteins on DEAE-cellulose (Whatman DE-52). Proteins obtained by this procedure were pooled, chromatographed on Sephadex G-75 equilibrated in 0.05 M ammonium bicarbonate for buffer exchange, and lyophilized.

Electrophoresis

Proteins were analyzed by sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) (10 and 3% (w/v) total acrylamide for the running gel and the stacking gel, respectively, with 2.5% bisacrylamide in each) using the buffer system of Laemmli (1970).

Amino Acid Analysis

The amino acid composition of purified proteins was determined by the method of Spackman et al. (1958). Prior to analysis, protein samples were oxidized with performic acid at 4°C prior to 24-h hydrolysis at 110°C. One crystal of phenol was added before acid hydrolysis. Serine was increased by 10% (w/v) and threonine by 5% to compensate for destruction by acid. Half-cystine was determined as cysteic acid.

Assay for Protein Concentration

Concentrations of proteins used as standards in the ELISA were estimated by determining the protein sulfhydryl content in the standard stock solution by the method of Grassetti and Murray (1967). Minor modifications of this assay included (1) the use of 50 mM phosphate ($NaPO_4$) buffer, pH 7.5, with 0.1 g EDTA/mL, rather than Krebs–Ringer phosphate buffer, and (2) the addition of 0.1 mL of 20 mM Tris–HCl, pH 8, the protein solvent, to the reagent blank. The value for protein sulfhydryl content was corrected for the half-cystine value obtained from amino acid analysis of the protein and expressed as micrograms or micromoles of protein.

Preparation of Antibodies

Goats were challenged initially with subscapular injections of about 500 μg of $HgBP_{20}II$ (see Results for description) suspended in Freund's complete adjuvant at 1 mL/1.5 mL protein to adjuvant ratio. Booster injections (about 100 μg of protein at the same protein to adjuvant ratio as above) were given at monthly intervals. The presence of specific antibodies was monitored by the Ouchterlony double gel diffusion assay (see below for method).

The immunoglobin-G (IgG) fraction from goat antiserum was purified by precipitation with ammonium sulfate and ion-exchange chromatography, using Whatman DE-52 equilibrated with 0.01 M sodium phosphate, pH 6.6 (Deutsch 1967). This fraction was checked by Ouchterlony analysis and immunoelectrophoresis using rabbit anti-whole goat serum and horse anti-goat IgG (Garvey et al. 1977). The protein concentration of the IgG stock solution was estimated by the method of Bradford (1976) with goat IgG (Sigma Chemical Co.) as standard.

Double Gel Diffusion Assays for Detection of Metal-Binding Proteins

The presence of precipitating antibodies and specificity of the Ag–Ab reaction were tested by the method of Ouchterlony as described by Garvey et al. (1977). Assays were conducted at room temperature on microscope slides coated with 3 mL of 1.5% (w/v) DIFCO Noble agar, 2% (w/v) polyethylene glycol 8000, 0.15 M NaCl, 0.05% (w/v) NaN_3, and 20 mM Tris–HCl, pH 8.4. After development of precipitation lines, the slides were sequentially washed in 0.3 M NaCl, 0.15 M NaCl, and 0.5% (v/v) glycerol, dried; stained with 0.1% (w/v) amido black in 7% (v/v) acetic acid, destained in 7% acetic acid, 0.5% glycerol, and photographed.

ELISA

The ELISA used in this study was an indirect, competitive enzyme immunoassay run under nonequilibrium conditions. The anti-(mussel HgBP) IgG described above was used as the primary antibody. A rabbit anti-goat IgG conjugated to horseradish peroxidase was used as the secondary antibody. The procedure followed protocol outlined by Voller et al. (1979) and Signorella and Hymer (1984):

(1) Reference antigen was immobilized on wells of microtiter plates (Dynatech Immulon 2) by adding 100 μL of a known amount of antigen dissolved in plate-coating buffer (PCB: 0.1

M sodium carbonate, pH 9.6) to each well, incubating for 18 h at 4°C, and washing with phosphate buffer solution (PBS) – Tween (0.01 M phosphate, 0.15 M NaCl, pH 7.6; 0.0125% (v/v) Tween-20).

(2) Unbound sites in each well were coated to block nonspecific binding of antibodies in subsequent steps by adding 200 μL of 1% (w/v) DIFCO gelatin dissolved in PBC, incubating for 4 h at room temperature, and washing with PBS–Tween.

(3) Solutions containing competing antigen and primary antibody were prepared and then added to microtiter plates. Concurrently with step 1, 100 μL of each of appropriate dilutions of unknowns or standard amounts of antigen and primary antibody solution were mixed in microcentrifuge tubes, incubated for 20 h at 4°C, and centrifuged for 1 min (Beckman Microfuge B). For preparation of standards, the stock antigen solution was dissolved in 20 mM Tris–HCl, pH 8.6, 4°C, at 106 μg/mL (8.21 nmol/mL). This solution was diluted with PBS–Tween to make working dilutions of standards. Unknown solutions and the primary antibody stock solution (89.2 mg IgG/mL) were also diluted with PBS–Tween. Following centrifugation of the above mixture for removal of precipitable complexes, the supernatant was added to microtiter plates after completion of step 2. The plates were then incubated for 18 h at 4°C and washed with PBS-Tween. Unbound antibody that remained in the supernatant was available to bind with the immobilized reference antigen during the incubation.

(4) Primary antibody that had bound to the reference antigen was reacted with the secondary antibody by adding 100 μL of the HRP-conjugate diluted in PBS-Tween to each well, incubating for 2 h at room temperature, and washing with PBS-Tween, followed by deionized water.

(5) The HRP activity of the bound conjugate was determined by adding 100 μL of substrate (0.1% urea peroxide; 0.4 mg *O*-phenylenediamine/mL as chromophore) in 0.1 M sodium citrate buffer, pH 4.5, to each well and incubating for 30 min at room temperature. After adding 100 μL of 2.5 M H_2SO_4 to stop the reaction, the absorbance was measured at 490 nm with a microtiter plate reader (BioTek 307).

Preliminary experiments in which various combinations of bound and competitive antigen, antibody, and conjugate concentrations were tested resulted in selection of the following for assay conditions: 106 ng of reference antigen added to each well, 0.106–106 ng of competing antigen for the standard curve, 268 ng of goat anti-(H_gBP_{20}II) IgG, and 1:500 dilution of the rabbit anti-(goat IgG)–HRP conjugate.

Conjugate and HRP substrate were purchased from Dynatech. Other reagents were purchased in Kit form from Dynatech or were prepared from reagent grade chemicals. All standards and samples were run in triplicate. The mean was used in subsequent computations. Each plate contained three replicate wells in which no competing antigen was added in step 3 and three wells in which both reference antigen in step 1 and competing antigen in step 3 were omitted. The former values represented positive controls assigned 100% absorbance values for the assay, while the latter represented negative controls assigned 0% absorbance for nonspecific binding of antibodies and reagents. Standard curves were constructed by plotting logit Y versus $\log_{10}$ standard antigen concentration, where logit Y = ln ($Y/1 - Y$), $Y = B/B_0$, B = sample absorbance minus 0% absorbance, and B_0 = 100% absorbance minus 0% absorbance.

Tested as unknown samples were cytosolic extracts of gills of control mussels and those exposed for 28 d to concentrations of 0.05, 0.1, 0.5, and 5 μg Hg/L, 1, 10, and 50 μg Cd/L, 5 μg Cu/L, and 10, 50, and 250 μg Zn/L, all administered as chloride salts in a flowing seawater system. Gills of five individuals were pooled for analysis. Extracts, which were obtained after 80 000 × *g* centrifugation of tissue homogenates as described above in the protein isolation protocol, required dilution (with PBS) prior to assay in the ELISA. Concentrations of metals in these gills were reported as part of an earlier study (Roesijadi and Fellingham 1987) and were used here for comparison with metal-binding protein values.

Commercially available rabbit MT (Sigma Chemical Co.) was also tested for cross-reactivity.

Results

The major low molecular weight, mercury-binding protein peak in mussel cytosol eluted at a position of about 20 kDa on Sephadex G-75. This was twice that expected for monomeric MT, which normally migrates at about 10 kDa on Sephadex. Previous work (Roesijadi 1986) indicated that this peak contains dimers of metal-binding proteins, since reduction with 2-mercaptoethanol shifted the elution position to about 10 kDa. Proteins in this study were isolated without the benefit of reduction, since it was felt that the dimeric form may reflect the natural state of the protein in these samples and reduction with mercaptoethanol at a concentration sufficiently high to dissociate the dimer (1% 2-mercaptoethanol) removes about 50% of the bound mercury (Roesijadi et al. 1982). The 20-kDa peak further separated into three protein peaks when subjected to anion-exchange chromatography with DEAE-cellulose (Fig. 1). One of these peaks, designated $HgBP_{20}$II was rechromatographed on Sephadex G-75, partially characterized, and used as antigen for development of antibodies and immunochemical detection procedures.

SDS–PAGE of $HgBP_{20}$II reduced with 1% (v/v) 2-mercaptoethanol resulted in separation of the protein into two bands reflective of either two proteins or the subunits of a heterodimer (Fig. 2). The latter was consistent with previous findings noted above that this protein exists as dimers that can be dissociated with mercaptoethanol treatment.

The amino acid composition of $HgBP_{20}$II resembled that of an MT with high cysteine (26%) and glycine (17%) contents (Table 1). Lacking were aromatic residues, histidine, and methionine. From the amino acid analyses, it was estimated that the unreduced protein had a minimum metal-free molecular mass of 12.9 kDa, which is approximately twice the expected value for metallothionein. Analysis of the protein for mercury indicated 12 g-atoms Hg/dimer, 6 g-atom/subunit if mercury is assumed to be distributed evenly between two subunits. The cys:Hg ratio was 2.4.

Injection of this protein into goats yielded antisera of suitable titer 3 mo after initial injection. Ouchterlony analysis of the antiserum against the immunizing antigen resulted in a single precipitation line. Preimmunization antiserum was not reactive. The purified IgG fraction containing goat anti-($HgBP_{20}$II) formed single precipitation lines when reacted against horse anti-goat IgG and rabbit anti-whole goat serum in Ouchterlony assays and immunoelectrophoresis.

Ouchterlony analysis of the IgG fraction obtained above showed a single precipitation line between it and $HgBP_{20}$II, the primary antigen, and cross-reactivity with $HgBP_{20}$I and $HgBP_{20}$III (Fig. 3). There was a reaction of identity between $HgBP_{20}$II and $HgBP_{20}$I and one of partial identify between $HgBP_{20}$II and $HgBP_{20}$III. These findings indicated that anti-

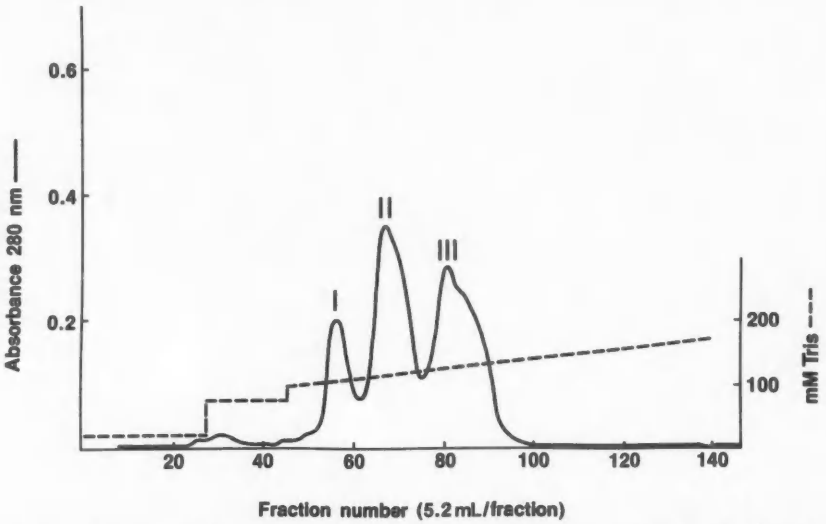

FIG. 1. Anion-exchange chromatography (Whatman DE-52) of $HgBP_{20}$ originally isolated by Sephadex G-75 chromatography. The proteins were eluted using a Tris gradient as shown in the figure. Major peaks are mercury-binding proteins designated $HgBP_{20}I$, II, and III. The chromatographic profile for mercury (not shown) paralleled that of the 280-nm absorbance. $HgBP_{20}II$ was used as antigen in subsequent development of antibodies against mussel metal-binding proteins.

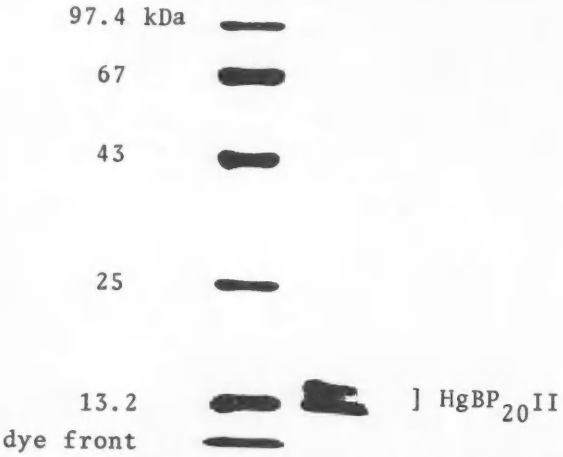

FIG. 2. SDS–PAGE (using buffer system of Laemmli 1970) of $HgBP_{20}II$ in Fig. 1. The separation gel was 10% total acrylamide and the stacking gel was 3% total acrylamide, with 2.5% bisacrylamide in each. Standards: 97.4 kDa, phosphorylase b; 67 kDa, bovine serum albumin; 43 kDa, ovalbumin; 25 kDa, chymotrypsinogen; 13.2 kDa, myoglobin; and dye front which co-migrated with aprotinin (6.5 kDa).

genic sites were completely shared by II and I, but not by II and III. Ouchterlony analysis also showed that the antibodies were reactive with cytosolic extracts of mussels, but not cross-reactive with $HgBP_{10}$ and high molecular weight cytosolic proteins (both isolated from mussel cytosol by Sephadex G-75 gel chromatography; $HgBP_{10}$ occurs as a shoulder on the $HgBP_{20}$ peak (Roesijadi 1986), rabbit MT obtained from Sigma Chemical Co., and cytosolic extracts of the liver of the white rat exposed to cadmium.

TABLE 1. Amino acid composition of $HgBP_{20}II$.

Amino acid	Residue percent	Nearest integer[a]
Asx	9.2	10
Thr	7.1	8
Ser	9.4	10
Glx	4.2	5
Pro	4.1	5
Gly	16.7	19
Ala	4.4	5
Cys/2	26.3	29
Val	3.9	4
Met	0.3	0
Ile	2.8	3
Leu	0.9	1
Tyr	0.3	0
Phe	0.3	0
His	0.4	0
Lys	7.9	9
Arg	1.8	2
Total		110[b]

[a]Calculated on the basis of one leu per molecule.
[b]Metal-free minimum molecular mass = 12.9 kDa.

Initial screening for reproducibility of antigen binding to microtiter plates during development of the ELISA indicated no significant difference among plates and an average coefficient of variation of 3.7% for all 96 wells on a single plate.

The results using known amounts of antigen under the conditions described above are shown in Fig. 4. A plot of B/B_0 versus log antigen dose indicated a sigmoid dose–response relationship (Fig. 4a). Logit Y transformation of B/B_0 (Fig. 4b) linearized the intermediate portion of the curve with 1.06 and 21.2 ng approaching the lower and upper limits of the linear range, respectively. These values corresponded to 0.082 and

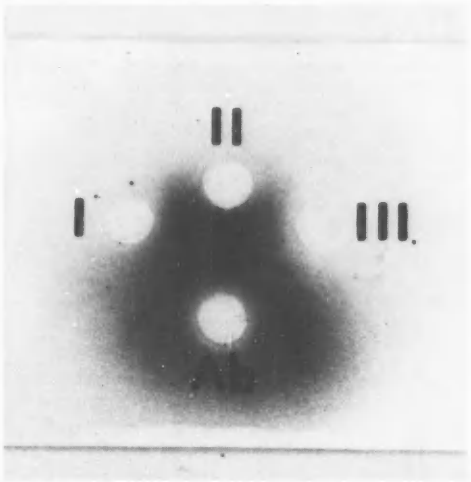

FIG. 3. Ouchterlony double gel diffusion assays of anti-($HgBP_{20}II$) (Ab) against $HgBP_{20}I$, II, and III. $HgBP_{20}II$ is the antigen used to induce antibodies. A reaction of identity between I and II and one of partial identify between II and III are observed.

1.642 pmol of $HgBP_{20}II$ using the dimer mass of 12.9 kDa for calculations.

$HgBP_{20}I$ and III were cross-reactive in the ELISA, while rabbit MT was not. The assay was sufficiently sensitive for detection of the basal levels in control gills, which were found to contain 0.5 μg metal-binding protein/g wet wt. Under conditions in which mercury exposure resulted in mercury accumulation and induction of metal-binding proteins (i.e. at exposures of 0.05–5 μg Hg/L), linearity between log metal-binding protein and log tissue mercury concentrations was observed (Fig. 5). The highest exposure concentration of 5 μg Hg/L resulted in 1780 μg metal-binding protein/g, indicating considerable capacity for induction by this metal. The curve approached an asymptote as the concentration of mercury in the gills approached the low concentrations in controls (Fig. 5). The basal levels of metal-binding proteins detected in these samples did not exist as mercury-binding proteins, since no detectable mercury was bound to the metal-binding proteins of controls (Roesijadi and Fellingham 1987). In contrast with cadmium, the metal was bound to the metal-binding proteins of controls (Roesijadi and Fellingham 1987), and the observed linearity (Fig. 5) also included the value of control mussels. Two hundred and fifty-seven micrograms of metal-binding protein per gram was detected at the highest exposure concentration of 50 μg Cd/L. Gills of mussels exposed to 5 μg Cu/L contained 3.7 μg metal-binding protein/g, a 7.4-fold increase when compared with controls. Those from mussels exposed to even extremely high concentrations of zinc contained only low levels of the proteins. Thus, at zinc exposure concentrations of 10, 50, and 250 μg Zn/L, metal-binding proteins only measured 1.3, 0.9, and 2.6 μg protein/g, respectively. This compared with total tissue zinc concentrations of 10.8, 11.6, and 22.2 μg Zn/g, respectively (Roesijadi and Fellingham 1987), with the concentrations in controls at 9.2 μg Zn/g. Zinc exposure resulted in little zinc bioaccumulation in gills, and this was reflected in the low levels of metal-binding proteins.

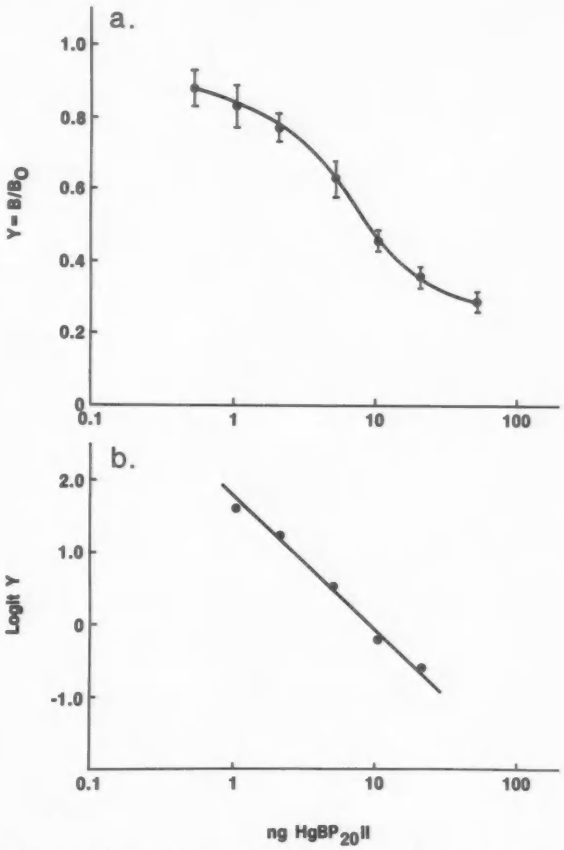

FIG. 4. ELISA standard curves with (a) $Y = B/B_0$) versus standard antigen concentrations and (b) logit Y versus standard antigen concentrations. $HgBP_{20}II$ is standard antigen, B is the background-corrected absorbance of standard or sample, and B_0 is the corrected absorbance of a positive control.

Discussion

A mercury-induced, MT-like protein from a marine bivalve mollusc (*M. edulis*) was isolated for use as antigen in development of antibodies and immunochemical detection procedures. This protein, one of three metal-binding protein peaks obtained after anion-exchange chromatography, was similar in molecular mass (George et al. 1979; Frazier et al. 1985) and amino acid composition (Frazier et al. 1985) to cadmium-binding proteins isolated from *M. edulis*. The amino acid composition was also similar to that of cadmium-binding proteins of the oyster *Crassostrea virginica* (Frazier et al. 1985). In addition to the usual characteristics of high numbers of cysteinyl residues and lack of aromatic amino acids, the mussel and oyster metal-binding proteins have in common a high number of glycine residues, a feature that may be representative of some molluscan variants of MTs. Based on recently published recommendations for nomenclature of MTs (Fowler et al. 1987), the proteins can be classified as MT.

Cross-reactivity of the antibodies against $HgBP_{20}II$ with the two other mercury-binding protein peaks, $HgBP_{20}I$ and $HgBP_{20}III$, and with metal-binding proteins induced by cad-

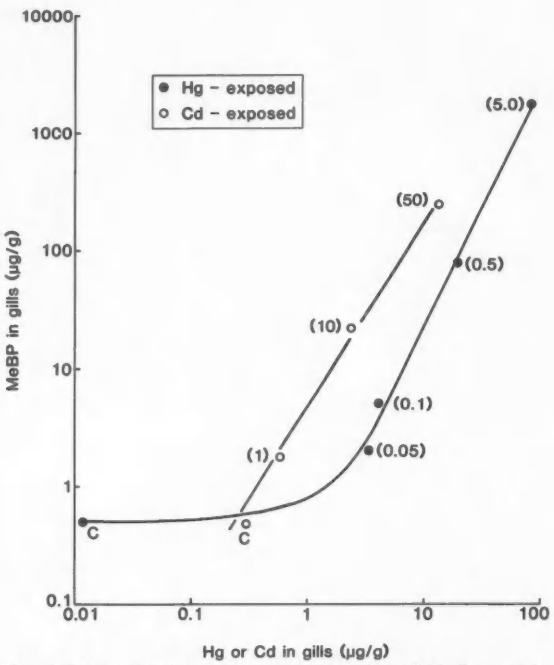

FIG. 5. Relationship between concentrations of metal-binding proteins (MeBP) and concentrations of mercury or cadmium in gills of mussels. Metal-binding proteins were estimated by ELISA. Mussels were exposed to mercury or cadmium for 28 d to the concentrations (µg/L; C = unexposed or control) shown in parentheses on the graph. With both mercury and cadmium, a linear log–log relationship was observed when there was evidence that the metal was bound to the proteins. In the case of mercury, metal-binding proteins of control mussels were not associated with mercury.

mium and copper supported their general suitability as reagents to quantify metal-binding proteins of mussels, although the lack of visible reactivity with $HgBP_{10}$ in Ouchterlony assays indicated that a small proportion of the total metal-binding proteins may not be detected in the ELISA. Roesijadi (1982) and Frazier et al. (1985) showed that metal-binding proteins corresponding to 20 kDa are the predominant forms in mussel tissue, with the 10-kDa forms representing a minor fraction. The lack of cross-reactivity with rabbit and rat MT in Ouchterlony assays is consistent with earlier observations (J.S. Garvey, Department of Biology, Syracuse University, Syracuse, NY, pers. comm.) that antibodies against mammalian MTs were not cross-reactive with mercury-binding proteins obtained from mussels. Differences in protein structure which are evident in the respective amino acid compositions may explain this lack of cross-reactivity (see Klauser et al. 1983 for amino acid composition of rabbit MT).

The sensitivity of the ELISA described here (working range of 1.06–21.2 ng; 0.082–1.6 pmol) was in the same general range as the radioimmunoassay described by Garvey et al. (1982) for mammalian MT. It was capable of detecting basal levels of metal-binding proteins in mussels. The sensitivity of the ELISA also compared favorably with other methods for the direct assay of MTs such as the isotope saturation and polarographic assays (Olafson and Sim 1979; Eaton and Toal 1982; Onosaka and Cherian 1982; Thompson and Cosson 1984). Immunochemical assays for MT offer the advantage of high specificity when compared with the other assays, although the nature of the specificity must be carefully considered, since some antibodies against MT, including those described here, are restricted in cross-reactivity with similar molecules.

The values for metal-binding proteins in gills of *M. edulis* were in the same general range of values obtained for livers of metal-exposed and unexposed fish by polarography (Roch et al. 1982) and rats by Hg and Cd radioassay and radioimmunoassay (Waalkes et al. 1985). Additionally, in the cases of exposure of mussels to mercury and cadmium, there was a direct relationship between the amount of metal-binding proteins in gills and the amount of metals in gills. This relationship could be described by the formula $Y = aX^b$, where Y = concentration of metal-binding protein and X = concentration of mercury or cadmium in the gill. A similar logarithmic relationship has been observed with MT and cadmium concentrations in human urine (Chang et al. 1980) and MT and cadmium and copper concentrations in rainbow trout (*Salmo gairdneri*) liver (calculated from Roch et al. 1982). Exposure to 5µg Cu/L resulted in a 7.4-fold increase in metal-binding proteins. With zinc, however, increases in metal-binding proteins as a result of exposure were minimal if at all. This observation was consistent with the report that zinc exposure did not result in increased levels of zinc bound to these proteins (Roesijadi and Fellingham 1987). Thus, when compared at roughly equivalent exposure concentrations (i.e. 5 µg Hg, 10 µg Cd, 5 µg Cu, and 10 µg Zn/L), the relative inducibility of the mussel MTs by these metals could be ranked as Hg > Cd > Cu > Zn.

Acknowledgments

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Correspondence Between Ecoregions and Spatial Patterns in Stream Ecosystems in Oregon

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Whittier, T. R., R. M. Hughes, and D. P. Larsen. 1988. Correspondence between ecoregions and spatial patterns in stream ecosystems in Oregon. Can. J. Fish. Aquat. Sci. 45: 1264–1278.

Multivariate analyses of biotic assemblages and physicochemical measures, species richness, diversity, and composition were used to evaluate the robustness of Omernik's ecoregion classification for small streams in the eight ecoregions of Oregon. Clearest differences were between the montane and nonmontane regions. For the three nonmontane regions, ordinations of fishes, macroinvertebrates, water quality, and physical habitat measures show the clearest differences, with the Willamette Valley ecoregion being consistently most unlike all other regions. Differences between the Columbia Basin and High Desert regions were clearest for water quality and physical habitat measures and fish assemblages. Differences among the montane regions were subtle. Of these regions, the East Cascade Slopes showed the greatest variability, as shown by the ranges of ordination scores for fishes, water quality, and physical habitat. Regional patterns in periphyton assemblages were markedly different from the patterns in the other groups of variables. Ecoregions can be used as a broad-scale geographic framework for classifying streams. This framework provides managers of lotic resources a useful alternative to river basins.

On a utilisé des analyses multivariées d'assemblages biotiques, de quantifications physicochimiques et de données sur la richesse, la diversité et la composition spécifiques pour évaluer la résistance de la classification d'Omernik des petits cours d'eau des huit régions écologiques de l'Oregon. Les différences les plus évidentes ont été notées entre les régions subalpines et non subalpines. Dans le cas des trois régions non subalpines, l'ordination des données sur les poissons, les macroinvertébrés, la qualité de l'eau et l'habitat physique révèle les différences les plus évidentes; ainsi, la région écologique de la vallée de la Willamette est nettement différente des autres régions. Les différences entre les régions du bassin du Columbia et de High Desert étaient plus marquées dans le cas de la qualité de l'eau, de l'habitat physique et des assemblages de poissons. Par contre, les différences entre les régions subalpines étaient ténues. De ces régions, les pentes East Cascades ont montré la plus grande variabilité, telle que révélée par les variations des degrés d'ordination obtenus pour les poissons, la qualité de l'eau et l'habitat physique. Les caractéristiques régionales des assemblages de périphyton étaient nettement différentes des caractéristiques observées chez les autres groupes de variables. Les régions écologiques peuvent servir de base géographique à grande échelle pour la classification des cours d'eau, base qui servira aux gestionnaires des ressources lotiques comme une autre solution utile à la gestion des bassins fluviaux.

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Just as the development of a taxonomic classification system proved a powerful tool for biologists, a river classification, however complex and elusive, has tremendous potential for researchers and managers of lotic resources. Classifications provide a common language for communication and a conceptual framework to organize component parts of complex systems. There have been many attempts to produce a stream classification. (See Hawkes (1975) for a brief history of lotic system classifications, especially as related to zonation studies.) The River Continuum Concept (RCC) (Vannote et al. 1980) implied a classification based on stream size or location within a stream system.

A major criticism (perhaps misunderstanding) of the RCC centered on the differences in streams in various geographic areas (e.g. Winterbourn et al. 1981). The original proponents of the RCC (Minshall et al. 1983, 1985) and others (e.g. Statzner and Higler 1985) have stated that regional differences among streams are a predictable and integral part of the RCC. A number of stream classification proposals have emphasized the importance of these geographic differences (Lotspeich and Platts 1982; Brussock et al. 1985; Frissell et al. 1986), and Culp and Davies (1982) suggested linking the RCC with such a scheme. However, with the exception of Brussock et al. (1985), none of these proposals offers an explicit geographic model (i.e. a map) to be evaluated.

A regional land classification, by itself, can provide a useful framework for studying and managing streams in different geographic areas. Given the linkage between stream characteristics and the surrounding land (Likens and Bormann 1974; Hynes 1975), grouping regions of similar land types should also group

similar streams (at some level of resolution) and thereby reduce overall variability (Warren 1979). There are several approaches to developing these geographic frameworks. To choose among the several geographic models, it is useful to know how the classifications were developed and their limitations related to intended uses.

Ichthyologists have explained fish distributions chiefly through use of drainage basins (e.g. Miller 1958) and physiographic regions (e.g. Trautman 1981; Pflieger 1971). The interconnectedness of waters in a river basin makes basins appropriate units for fish distribution. But basins do not account for changes in water characteristics derived from changes in the surrounding land. Legendre and Legendre (1984) demonstrated that in Quebec, climate, geomorphology, and vegetation patterns were more useful than river basins in explaining fish distributions because of postglacial changes in drainage patterns. Physiographic regions, based on the genesis and evolution of land forms, account for many of the causal factors in aquatic ecosystems, but do not necessarily correspond with others such as soil, climate, and land use (Omernik 1987). Hughes et al. (1987) found that physiographic regions were less useful than Omernik's (1987) ecoregions or river basins for explaining historical fish distribution patterns in Oregon. The weakness of basins, physiographic regions, or single landscape variables is that they do not integrate a broad spectrum of causal factors.

Another method for developing aquatic regions is an a posteriori inductive approach using a large volume of site-specific data. These data can be subjected to cluster and discriminant analyses to derive spatial patterns and suggest causal relationships. This was the approach taken by Pflieger et al. (1981), Legendre and Legendre (1984), and Hawkes et al. (1986). Although realistic regions can be produced, this process is limited by its data-intensive nature, lack of extrapolability to other variables, and sensitivity to data noise.

In the last decade there has been growing interest in a synoptic, or holistic, approach to developing ecological regions (Rowe and Sheard 1981; Bailey 1983). Essentially, this is an a priori deductive method that looks for broad-scale patterns in several mapped land and climate characteristics that are functionally related to the ecosystems of interest (Warren 1979). Spatial patterns in these separate maps are synthesized to delineate ecological regions. Two notable examples are the Land Resources Regions and Major Land Resource Areas of the United States (USDA Soil Conservation Service 1981) based on soil, climate, water availability, vegetation type, and land use, and Bailey's (1976, 1981) hierarchical map of ecoregions of North America based primarily on climate at the coarser levels of resolution and potential natural vegetation at the finer levels.

More recently, Omernik compiled maps of ecoregions of the United States (Omernik 1987) and ecoregions of the Pacific Northwest (Omernik and Gallant 1986) by noting regional similarities in a combination of terrestrial characteristics that integrate features associated with differences in streams. He based his regional framework primarily on state and national soil maps and national maps of land-surface form, potential natural vegetation, and land use (Hammond 1970; Kuchler 1970; Anderson 1970). The ecoregion maps grew from an effort to classify streams for water resource management that originally used Warren (1979) as a logical framework and Bailey's ecoregions as a spatial framework (Hughes and Omernik 1981; Omernik et al. 1982).

Rowe and Sheard (1981) and Bailey (1983) proposed that land classification systems be treated as geographic models derived from ecological theory and that these models must be evaluated. To our knowledge, few studies of ecoregion models have occurred. Olson et al. (1982), using discriminant analyses, found a high degree of separability at the section level in Bailey's Laurentian mixed forest province with patterns of vegetation, soil, and climate. Spatial patterns in water chemistry and fish distributions have been shown to correspond to Omernik's ecoregions in small streams in Arkansas (Rohm et al. 1987) and Ohio (Larsen et al. 1986; Larsen et al. 1988; Whittier et al. 1987), as do fish in Oregon rivers (Hughes et al. 1987) and trophic states in Minnesota lakes (Heiskary et al. 1987).

Based on these studies, we feel that Omernik's ecoregions currently represent the most appropriate framework for classification of lotic systems. The purpose of this study is to further evaluate Omernik's classification by determining how well spatial patterns in several characteristics of small stream ecosystems correspond with the eight ecoregions of Oregon (Fig. 1). To do this, we examined patterns in fish, periphyton, and macroinvertebrate assemblages and the physical habitat and water quality of 49 small streams in and around Oregon.

Methods

Site Selection

Each ecoregion in Omernik's classification is divided into two parts: (1) The "most typical" portion shares all four land characteristics that typify the ecoregion. This area may be considered as the core of the region. (2) The remainder, the "generally typical" portion, shares most but not all of the same characteristics (Omernik and Gallant 1986). These areas usually represent transition zones among the cores of the ecoregions. For each of Oregon's eight ecoregions (Fig. 1), we selected six watersheds wholly included within the core areas of that ecoregion. We also selected six watersheds in the transition areas of each region.

Following the methodology of Hughes et al. (1986), we sought wadable stream sites that were minimally impacted by human activity and as broadly distributed as possible throughout the ecoregions. Whenever possible, we chose sites that were not below point sources of pollution or clear-cuts and were not heavily impacted by agriculture, particularly livestock use. However, few of these sites could be considered pristine.

Field Sampling

Each stream site was sampled once for all variables by one of three field crews 17 July to 9 September 1984. One site in the East Cascade Slopes ecoregion was dropped because of highly alkaline water from springs. Two additional sites were sampled in this ecoregion. At each site the crew selected three (contiguous in most cases) approximately 100-m-long reaches that encompassed complete sets of the characteristic stream forms for that site, e.g. pools, riffles, runs. Waterfalls and pools too deep to wade were excluded. To partially compensate for seasonal differences occurring over the course of our survey, we sampled the Willamette Valley ecoregion first, then the Columbia Basin and High Desert regions, and the mountainous regions last. Thus the mountain streams were sampled at their warmest.

Fish were collected from the three reaches using 3-mm-mesh dipnets and backpack or towed electroshockers producing

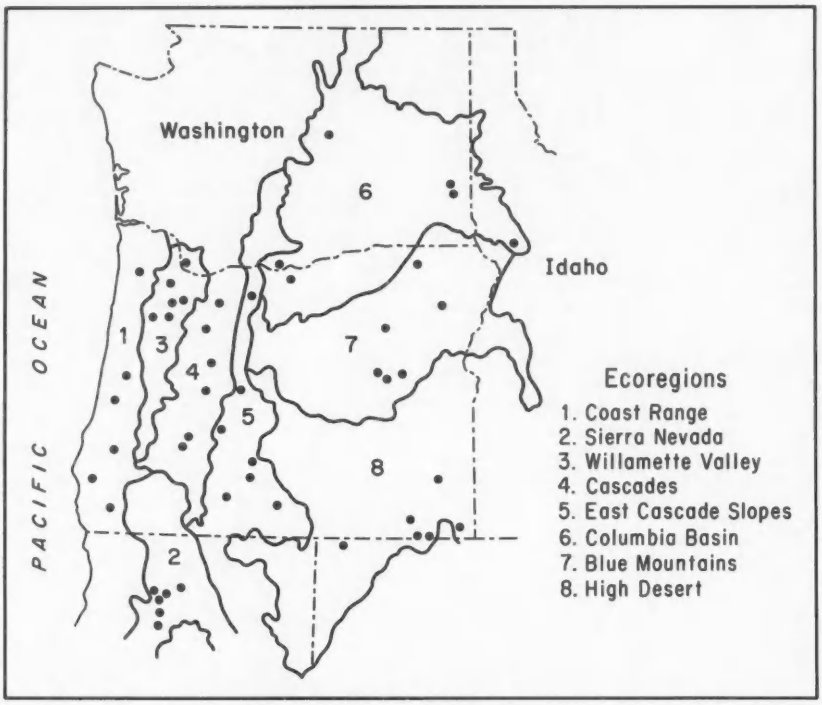

FIG. 1. Ecoregions of Oregon (USA) and nearby areas. Dots indicate sampling sites used in this study.

1.5–3 amps DC at 80–90 pulses per second. All fish per reach were held in a livewell to be identified, counted, and weighed.

Periphyton samples were scraped from large stable substrates in high- and low-velocity locations in each reach and preserved in a formalin – acetic acid – alcohol solution. Periphyton taxa were identified and enumerated. The relative abundances of the taxa in each sample were based on counts of approximately 500 cells (McIntire and Overton 1971).

Macroinvertebrates were sampled from coarse substrate in shallow, fast-flowing waters by the traveling kick net method using a 0.5-mm-mesh net (Pollard and Kinney 1979). At each site, three transects were sampled totaling approximately 12 m. Macroinvertebrate taxa from the preserved samples were identified to species level whenever possible and enumerated.

Water quality and physical habitat were evaluated at each site. Three midpool grab samples were taken. Dissolved oxygen (modified Winkler), alkalinity (fixed endpoint titration), conductivity and pH (electrometric analyses), and air and water temperatures were taken. One 200-mL water sample was preserved with $HgCl_2$ for laboratory analysis of nitrate-nitrite, ammonia, Kjeldahl nitrogen, orthophosphate, and total phosphorus using a Technicon auto analyzer, turbidity using a Hach turbidimeter, and total organic carbon using a Coulemetric carbon analyzer. A 100-mL sample was acidified with 2% concentrated HNO_3 for laboratory analysis of hardness using plasma emission spectroscopy of Ca and Mg. Blind duplicates, distilled water blanks, and standards were included for quality assurance. Chemical analyses followed U.S. Environmental Protection Agency standard methods (USEPA 1979).

Physical habitat was sampled at 10-m intervals along the fish sampling reaches. At each interval the following metrics were recorded: dominant riparian vegetation (tree, shrub, herbaceous, or absent); bank stability, bank rock content, and embeddedness classes estimated as 25, 50, 75, or 100%; stream depth; stream morphology described as pocket water, riffle, chute, run, glide, or pool; dominant stream substrate described as debris, mud (muck or silt), sand, gravel, cobble, boulder, or bedrock (or claypan); and concealment for fish described as undercut bank, overhanging vegetation, woody debris, boulders, macrophytes, turbidity (or turbulence), or anthropogenic structures.

Data Analyses

Our primary interest was to identify spatial patterns among the biota and physical/chemical parameters and to evaluate the correspondence between these patterns and the aquatic ecoregions as mapped. To elicit patterns in the three biotic groups sampled, we chose Detrended Correspondence Analysis (DCA), an ordination technique that examines the similarity of sites in species space by determining orthogonal axes that maximize variance of sites in species space. DCA reduces the "arch" effect and the compression of samples near the ends of the first axis which occur with other ordination techniques (Gauch 1982a).

We examined the location of sites based on species data along the first three DCA axes. Gauch (1982b) showed that most of the meaningful variability is accounted for by the first few ordination axes. We would expect that if no correspondence exists between the mapped regions and patterns in stream character, then regional sites would appear randomly scattered in species space. Conversely, if there were complete and exclusive correspondence between the ecoregions and stream character, we would see eight distinct regional groups in species space. We used a version of DCA called DECORANA (Hill 1979).

A major function of the ordination technique is as an exploratory tool (Gauch 1982a). Toward that end, for each of the three biotic groups, we ran several DCA's with $\log_{10}$-transformed abundances and presence/absence forms of the data. We compared ordinations of all sites, the ecoregion core sites only, dropping obvious outliers, and in the case of the fish data, dropping single species sites. To facilitate our study of spatial patterns and to compare ordinations, for each ordination we considered: Do sites of a region group together in species space? How distinct are regional groups? Do sites in the core portions form regional cores in species space? Are regional patterns consistent among the biotic and abiotic groups of variables? Are regional patterns consistent when ordinations of all sites are compared with those of the core sites alone? Are these patterns consistent across data transformations?

For macroinvertebrates and periphyton the level of taxonomic resolution reasonably attainable varies. Some groups may be easily identified only to order or family without considerable additional expenditure of effort. Also, the taxonomy of these groups is incomplete and in a general state of flux. These concerns caused us to examine all biotic groups at both the family and the lowest feasible taxonomic (species whenever possible) level of resolution.

Because of the complexity of the periphyton data, we used the Cornell Ecology Program TWINSPAN (Gauch 1982a) to hierarchically tabulate groups of sites and their characteristic taxa. Rather than identifying the numerically dominant taxa, the program chooses the indicator taxa that distinguish each group.

Principal Components Analysis (PCA) of the correlation matrix was used to ordinate the sites with respect to the water quality and physical habitat variables. The SAS Princomp procedure (SAS Institute, Inc. 1986) was used for these analyses. Proportional physical habitat measures were transformed using arcsin square root to achieve a more normal distribution (Sokal and Rohlf 1981). Chemical values were $\log_{10}$ transformed. As with the biotic data, we examined ordinations in several combinations of sites and variables (e.g. ionic strength measures only, nutrient measures only, both together, etc.) for regional patterns in the abiotic parameters among the sites.

We also examined the within- and among-region variation on a univariate basis. We analyzed regional patterns in individual water quality and physical habitat measures, and taxa and family richness and diversity using medians, ranges, and interquartile ranges (Reckhow 1980). Finally, to better understand which organisms are characteristic of the regions, we developed regional taxa signatures for the common fishes, macroinvertebrates, and periphyton. These signatures are analogous to the pollen diagrams used by paleontologists to demonstrate changes in plant communities over time. In this case, we wish to show spatial, rather than temporal, patterns.

Results and Discussion

For this paper, we present data from the 49 regional core sites, i.e. those sites that occur in the "most typical" areas of each ecoregion (Omernik and Gallant 1986). Regional differences are most clearly seen in these sites. Sites in the transitional areas were indeed transitional, blending characteristics of neighboring regions. For the biota, we present the ordinations derived from presence/absence data. This form showed the same general regional patterns as the abundance data, except for the fishes where the first axis of the abundance data ordination was dominated by one species (*Rhinichthys osculus*) masking other relationships.

Fish

We collected 38 fish species in 11 families. *Salmo gairdneri* was the only species taken at eight sites, five of which occurred in the Sierra Nevada ecoregion. The total number of species collected in each region ranged from 2 in the Sierra Nevada to 23 in the Willamette Valley (Table 1). Species diversities (H') ranged from 0.0 (in 5 regions) to 1.82 in a Cascades site. Except for the Sierra Nevada, regional median H' values varied little (0.45–0.96). Species richness ranged from 1 (at nine sites among five regions) to 14 at a Willamette Valley site. No introduced species were collected in the Sierra Nevada, Coast Range, or Columbia Basin ecoregions. All Willamette Valley sites had exotic species (Table 2).

The DCA ordination of the presence/absence data separated the montane ecoregions from the nonmontane regions along Axis I (Fig. 2). This axis also tended to separate the Blue Mountains region from the Coast Range, Sierra Nevada, and East Cascade Slopes. Axis II separated the Willamette Valley from the High Desert and Columbia Basin regions. Two of the High Desert sites (C in Fig. 2) are in the Columbia River drainage and had *Catostomus columbianus*, which is a characteristic fish of the Columbia Basin ecoregion (Wydoski and Whitney 1979; Hughes et al. 1987), along with *Richardsonius balteatus* and *Rhinichthys osculus*. The other High Desert sites drain to the interior. At one of the interior sites (at 250, 400 in Fig. 2), we collected only Lahontan cutthroat trout (*Salmo clarki henshawi*), a distinct subspecies endemic to the Great Basin (Behnke and Zarn 1976; Moyle 1976).

The fishes characteristic of each region are shown in Fig. 3. The four "high mountain" regions (on the left) had similar fish assemblages (also shown by their overlap in the ordination).

TABLE 1. Regional patterns in fish assemblage indices.

	Sierra Nevada	Blue Mountains	Cascades	East Cascade Slopes	Coast Range	High Desert	Columbia Basin	Willamette Valley
Species collected	2	9	11	5	7	9	8	23
Species richness[a]	1 (1–2)	3.5 (2–5)	2.5 (1–8)	2 (1–2)	3.5 (1–6)	2.5 (1–6)	4.5 (2–7)	6.5 (5–14)
Species diversity (H')[a]	0 (0.0–0.66)	0.96 (0.08–1.24)	0.67 (0.0–1.82)	0.45 (0.0–0.69)	0.81 (0.0–1.52)	0.51 (0.0–1.41)	0.75 (0.32–0.99)	0.81 (0.28–1.54)

[a]Regional median values with ranges in parentheses.

TABLE 2. Exotic fish species collected.

Ecoregion	Species	Number of sites
Blue Mountains	*Salvelinus fontinalis*	2
Cascades	*S. fontinalis*	1
East Cascade Slopes	*S. fontinalis*	2
High Desert	*S. fontinalis*	1
Willamette Valley	*Cyprinus carpio*	2
	Carassius auratus	1
	Fundulus diaphanus	1
	Gambusia affinis	2
	Lepomis macrochirus	4
	L. gulosus	3
	Micropterus salmoides	2
	Perca flavescens	1

Sites in these regions were dominated by *Salmo gairdneri* and typically had only a few other species, usually other salmonids and cottids. The Coast Range, which is an older, lower elevation mountain system, also had a montane fish assemblage along with *Oncorhynchus kisutch* and *Lampetra tridentata*. The Willamette Valley ecoregion had the greatest variety of fishes including several often associated with pools (Centrarchidae) and slow, turbid streams (Cyprinidae, Catostomidae) (Wydoski and Whitney 1979). Half of the sites in the Columbia Basin ecoregion had no salmonids. This region was characterized by cyprinids and catostomids. The High Desert sites also were dominated by minnows and suckers. Two High Desert sites had redband trout (*Salmo* sp.) which is either a subspecies of rainbow trout or a closely related species (Schreck and Behnke 1971; Moyle 1976; Gold 1977) which has adapted to survive warm temperatures.

Macroinvertebrates

We identified 300 macroinvertebrate taxa in 90 families. Regionally, there was a general inverse relationship between the total number of insect and noninsect taxa collected. The Sierra Nevada ecoregion had the highest number of insect taxa and lowest number of noninsect taxa; the reverse occurred in the Willamette Valley (Table 3). Richness ranged from 12 taxa at a Willamette Valley site to 69 at a Sierra Nevada site. Regional median richness values were lowest in the Willamette Valley (26) and Columbia Basin (27) and ranged from 36 to 49 in the other regions. Regional median diversity (H') values were also lowest in the Columbia Basin (1.8) and Willamette Valley (2.1) and ranged from 2.6 to 3.0 in the remainder (Table 3).

The DCA of the presence/absence data (Fig. 4) was representative of all versions of this analysis (abundance data, and at the family and genus level). The first axis separated the five mountainous regions from the others, although two East Cascade Slopes sites ordinated in the nonmontane area of Axis I. One Willamette Valley site that had almost pond-like conditions had a very high Axis I score (420). Removing this site as an outlier did not affect the regional patterns shown by DCA. Axis II tends to separate the Willamette Valley from the Columbia Basin and the High Desert ecoregions. The compact group formed by 29 of 31 sites in the montane ecoregions indicates a high degree of similarity in their macroinvertebrate assemblages relative to the other ecoregions.

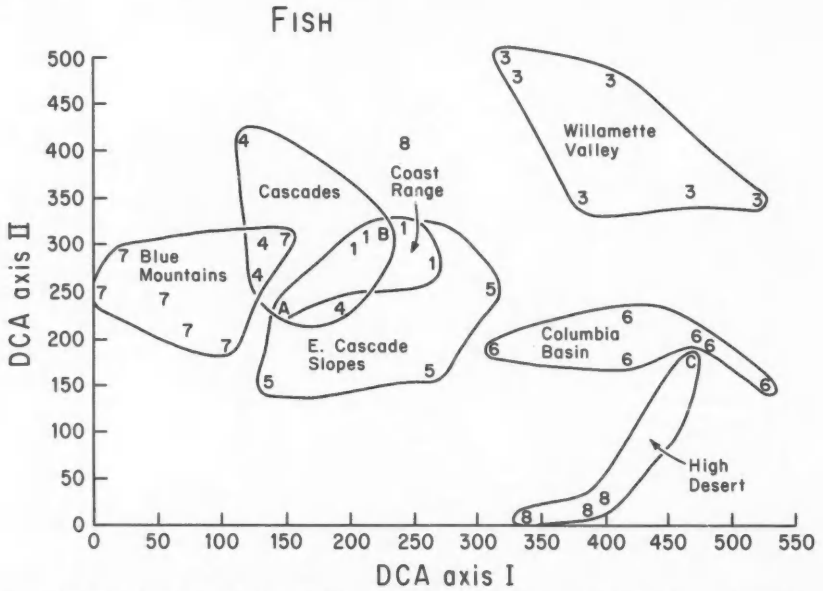

FIG. 2. Regional patterns in fish assemblages shown by DCA ordination of 49 sites, using species presence data. Lines circumscribing regions subjectively emphasize regional groups and do not imply any statistically tested differences. A = eight sites with 100% *Salmo gairdneri* (five in Sierra Nevada, one each in Cascades, East Cascade Slopes, and Coast Range). B = five sites with only *Salmo gairdneri* and *Cottus perplexus* (two in East Cascade Slopes, one each in Cascades, Sierra Nevada, and Coast Range). A and B include all Sierra Nevada sites. C = two High Desert Sites in the Columbia River drainage. Numbers correspond to ecoregions in Fig. 1.

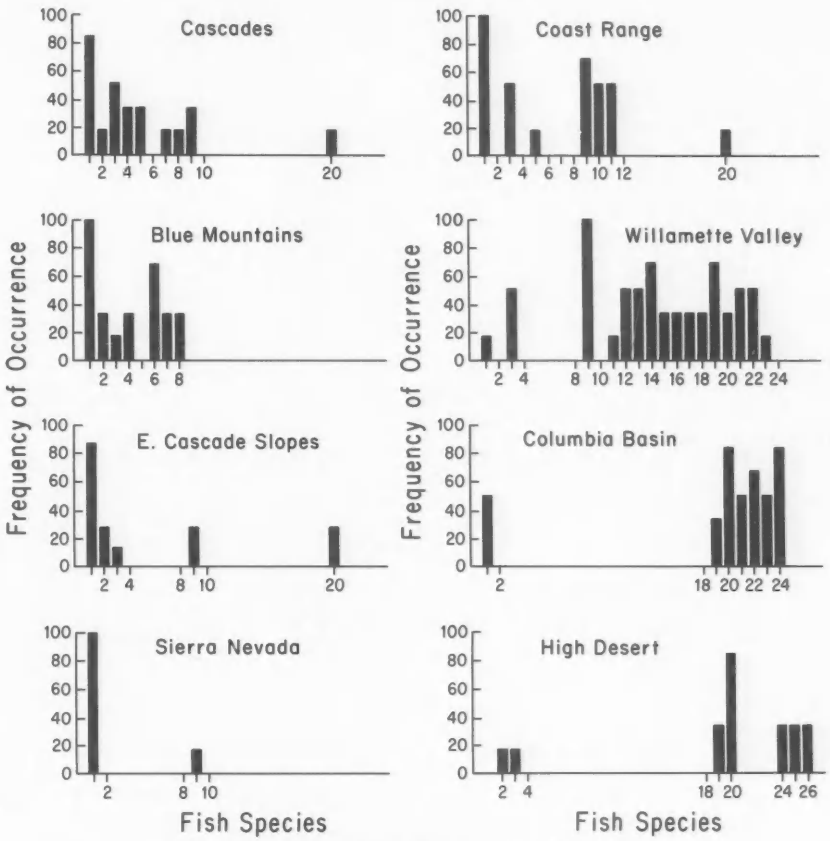

FIG. 3. Regional frequencies of occurrence of 26 common fish.

1. *Salmo gairdneri*
2. *Salvelinus fontinalis*
3. *Salmo clarki*
4. *Cottus bairdi*
5. *Cottus rhotheus*
6. *Salvelinus confluentus*
7. *Cottus beldingi*
8. *Prosopium williamsoni*
9. *Cottus perplexus*
10. *Lampetra tridentata*
11. *Oncorhynchus kisutch*
12. *Lampetra pacifica*
13. *Lepomis gulosus*
14. *Lepomis macrochirus*
15. *Micropterus salmoides*
16. *Cyprinus carpio*
17. *Gambusia affinis*
18. *Gasterosteus aculeatus*
19. *Richardsonius balteatus*
20. *Rhinichthys osculus*
21. *Catostomus macrocheilus*
22. *Ptychocheilus oregonensis*
23. *Acrocheilus alutaceus*
24. *Catostomus columbianus*
25. *Catostomus tahoensis*
26. *Salmo* sp. (redband trout)

To show some of the regional macroinvertebrate assemblage structure, we plotted the relative abundances of 17 common insect families and all noninsect taxa (Fig. 5). In general, the patterns in the five montane regions were similar. This would be expected based on their similar ordination scores. In only 2 of 31 sites in these regions did any family account for more than 50% of the macroinvertebrates. Noninsects accounted for ≤10% of the assemblages in 26 sites.

The patterns in the three nonmontane regions were noticeably different from the montane regions, and from each other. In the Willamette Valley, 3 of the 17 families were not collected, 5 other families were collected at only one site (with abundances < 1% of the individuals), and noninsects composed more than 21% of the collection in five of six sites. In the Columbia Basin, five families were not collected, three other families were collected at only one site (< 1% of the individuals), and four of six sites were dominated (relative abundances ≥50%) by one insect family or by noninsects. In the High Desert region, one insect family was not collected, four other families were rarely collected, and two of six sites were dominated by one family.

Periphyton

We collected 288 periphyton taxa in 37 families. The total number of taxa collected ranged from 67 in the Cascades to 129 in the Willamette Valley. Diversity (H') ranged from 0.46 in an East Cascade Slopes site to 3.49 in a Willamette Valley site

TABLE 3. Regional patterns in macroinvertebrate assemblage indices.

	Sierra Nevada	Blue Mountains	Cascades	East Cascade Slopes	Coast Range	High Desert	Columbia Basin	Willamette Valley
Insect taxa[a]	114	98	102	106	100	104	75	67
Noninsect taxa[a]	4	10	7	9	7	13	15	19
Taxonomic richness[b]	47 (41–69)	42 (40–60)	49 (44–53)	36 (28–41)	39 (21–49)	38 (26–45)	27 (20–34)	26 (12–35)
Diversity (H')[b]	2.8 (2.6–3.2)	2.7 (2.3–3.4)	3.0 (2.6–3.4)	2.6 (2.1–3.0)	2.9 (2.3–3.3)	2.6 (2.0–3.1)	1.8 (1.0–2.3)	2.1 (1.5–2.7)

[a]Regional totals of macroinvertebrate taxa collected.
[b]Regional median values with ranges in parentheses.

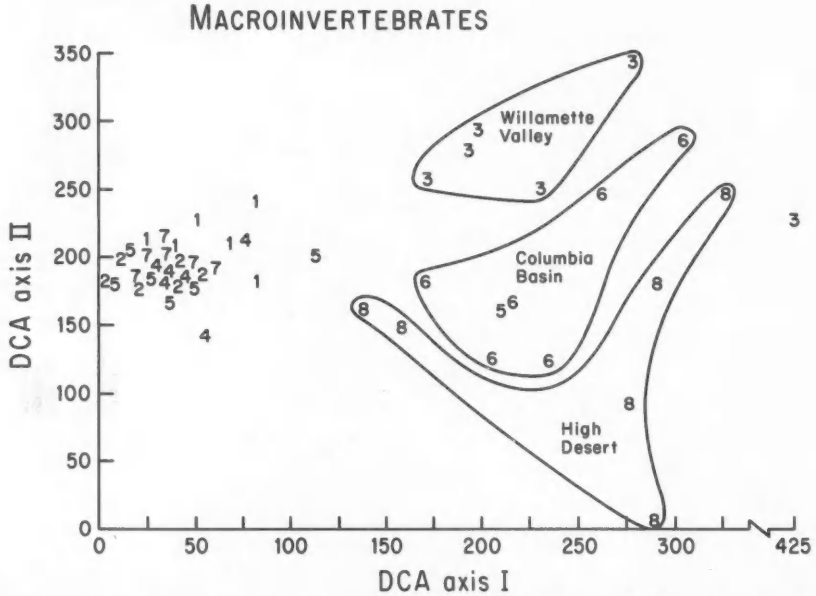

FIG. 4. Regional patterns in macroinvertebrate assemblages shown by DCA ordination of 49 sites, using species presence data. Numbers specify ecoregions as in Fig. 1.

(Table 4). Regional median H' values ranged from 1.86 in the Coast Range to 3.30 in the Willamette Valley. Richness ranged from 13 taxa at an East Cascade Slopes site to 71 in one Willamette Valley site. The lowest regional median richness occurred in the Sierra Nevada (26) and the Coast Range (26.5); the highest was in the Willamette Valley (61.5), with the others ranging from 30.5 to 46.

The DCA of the presence/absence data separated the Willamette Valley from all other ecoregions on Axis I (Fig. 6). Axis I also separated the Columbia Basin from the Blue Mountains and somewhat from the Coast Range. The East Cascade Slopes Axis I scores were also higher than those of the Blue Mountains. Axis II separated the "non-Willamette Valley" sites into two groups. The upper group of ecoregions share contiguous volcanic mountain ranges (the Cascades, Klamath, and Sierra Nevada). The lower group of regions, except for the Coast Range, are east of the former regions and in areas that tend to be warmer and drier.

The patterns of periphyton distribution were more complex than for the other biotic groups. Five taxa were widespread and among the dominants in most regions. Because the geographic pattern of sites grouped by TWINSPAN resembled the ecoregion classification, we used indicator species selected by the program (those species that distinguished the groups of similar sites) to show abundance patterns of regionally characteristic taxa (Fig. 7).

Physical Habitat

We combined physical habitat features rarely encountered with variables judged to have similar habitat functions (e.g. boulders with cobbles, runs with glides) to give 28 variables. The first three PCA axes accounted for 49.5% of the variance. The first axis accounted for 24.7% of the variance and separated the Willamette Valley and High Desert from the montane regions; the Columbia Basin and East Cascade Slopes regions were intermediate (Fig. 8). Axis II, accounting for 12.6% of the variance, separated the Willamette Valley from the High Desert and the Columbia Basin/East Cascade Slopes group. This axis also generally separated the Coast Range and the Sierra Nevada from the Blue Mountains. The third axis

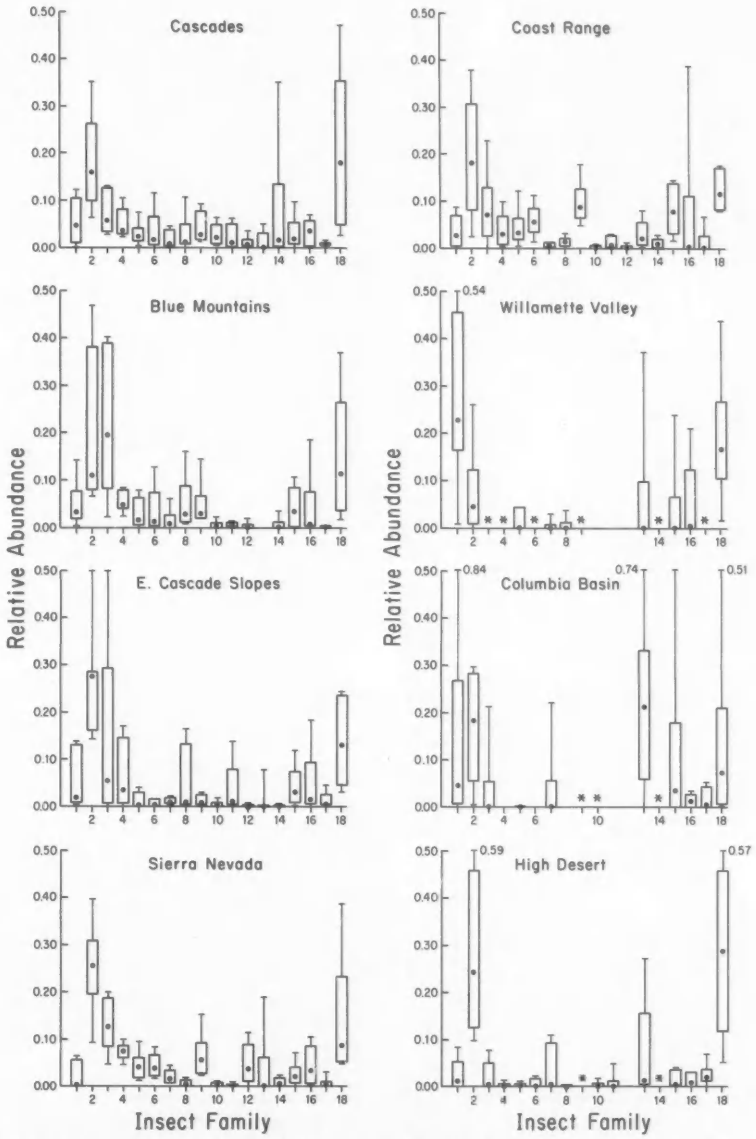

FIG. 5. Regional relative abundances of 17 common insect families and all noninsect taxa. Box plots summarize the relative abundance of each family at all sites in each region. The box plots include median (●), interquartile range (height of the box), and range (length of vertical line).

1. Noninsect taxa combined

Ephemeroptera
2. Baetidae
3. Heptageniidae
4. Ephemerellidae

Plecoptera
5. Nemouridae
6. Perlidae
7. Perlodidae
8. Chloroperlidae

Trichoptera
9. Rhyacophilidae
10. Glossosomatidae
11. Philopotamidae
12. Arctopsychidae
13. Hydropsychidae
14. Limnephilidae

Coleoptera
15. Elmidae

Diptera
16. Simuliidae
17. Tipulidae
18. Chironomidae

Asterisks indicate families collected at only one site in the region and which comprised <1% of the site's macroinvertebrate collection. Blanks indicate families not found at any sites in the region.

TABLE 4. Regional patterns in periphyton assemblage indices.

	Sierra Nevada	Blue Mountains	Cascades	East Cascade Slopes	Coast Range	High Desert	Columbia Basin	Willamette Valley
Taxa collected[a]	69	91	67	116	73	106	91	129
Taxonomic richness[b]	26 (17–33)	34.5 (25–49)	30.5 (22–39)	46 (13–50)	26.5 (24–36)	43 (30–58)	39 (33–47)	61.5 (44–71)
Diversity (H')[b]	2.01 (1.75–2.43)	2.39 (1.44–3.21)	2.18 (1.86–2.79)	2.75 (0.46–2.98)	1.86 (0.93–2.35)	2.57 (2.38–3.23)	2.67 (2.52–3.05)	3.30 (3.12–3.49)

[a]Regional totals of periphyton taxa collected.
[b]Regional median values with ranges in parentheses.

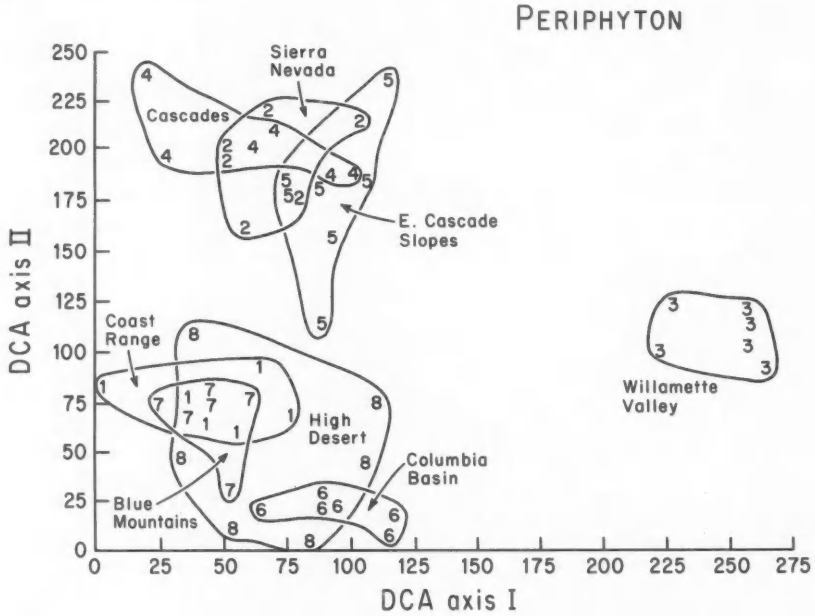

FIG. 6. Regional patterns in periphyton assemblages shown by DCA ordination of 49 sites, using species presence data. Numbers specify ecoregions as in Fig. 1.

which accounted for 12.2% was loaded by the bank stability and bank rock content variables and showed no regional patterns. Physical habitat variables that loaded >20% on PCA axes I or II are shown in Table 5.

Water Quality

All three of our measures of ionic strength (alkalinity, conductivity, hardness) were highly correlated ($r > 0.91$); the regional patterns in conductivity are representative (Table 6). Orthophosphate and total phosphorus were highly correlated ($r = 0.98$). Total phosphorus also correlated with turbidity ($r = 0.85$). Ammonia nitrogen concentrations were at the detection limits at most sites and were excluded from the data. The regional patterns for Kjeldahl nitrogen are generally representative of those for nitrate and nitrite. Total organic carbon was not well correlated with other measures of nutrient richness ($r < 0.35$) and had markedly different regional patterns (Table 6).

Because of the high correlation among variables, PCA Axis I accounted for 95.8% of the variability in the ionic strength measures. The nutrient richness variables tended to fluctuate more independently of each other. This lower correlation was reflected in the lower fraction of variability accounted for by the principal axes of the nutrient analysis, 63.8% for Axis I and 29.0% for Axis II.

The results of the two PCAs are summarized as a graph of ionic strength PCA I versus nutrient richness PCA I (Fig. 9). This graph shows the relationship between water quality of the sites and their ecoregions, particularly for the nonmontane regions. The Columbia Basin had enriched water with high ionic strength. The Willamette Valley streams were generally more enriched with somewhat lower ionic strength. The High Desert streams were intermediate between these regions and the montane regions. While considerable overlap among the latter group of regions occurred, some regional patterns were notable: the Sierra Nevada fell in the intermediate range for ionic strength but had barely detectable nutrient levels; the Blue Mountains were all nutrient poor and five of six sites had moderately low ionic strength; all Cascades sites were in the lower half of the

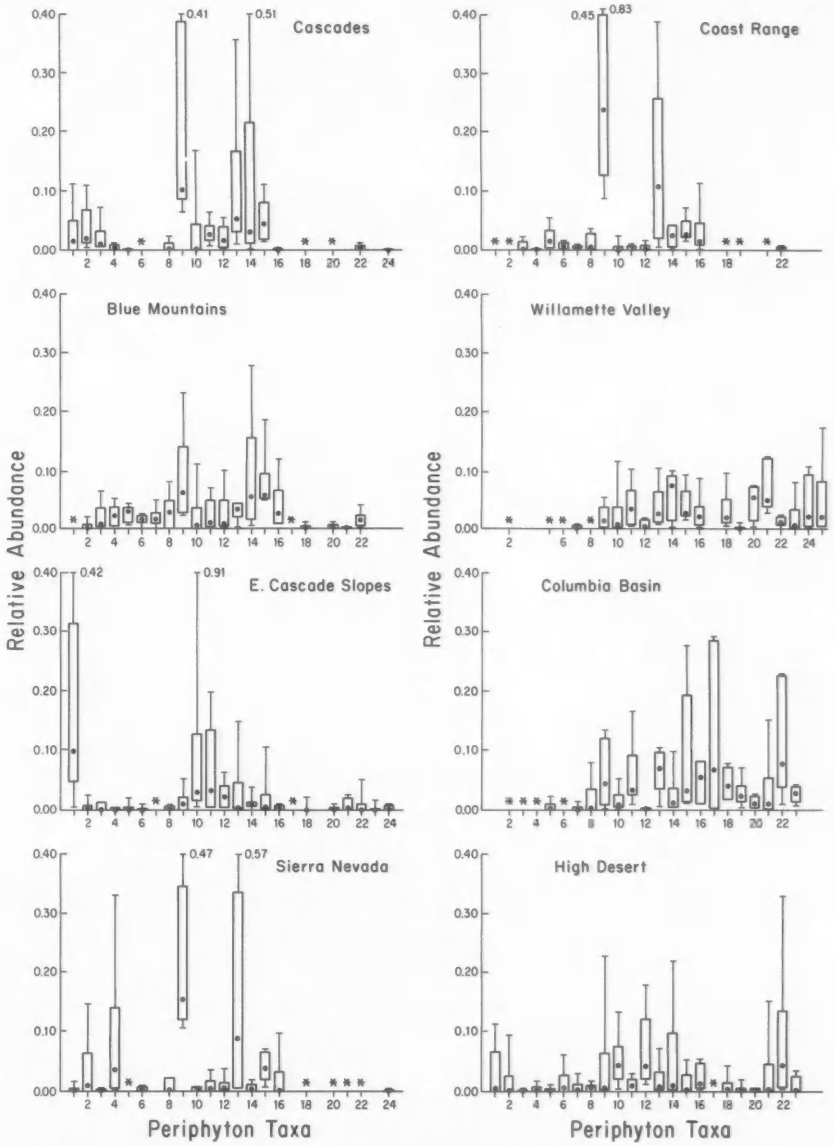

FIG. 7. Regional patterns of relative abundances of 20 periphyton taxa selected by the TWINSPAN program (indicator taxa) and five widespread abundant taxa. The box plots summarize the relative abundances of each taxon at all sites in each region. Box plots as in Fig. 5.

1. *Nostoc spp.*
2. *Gomphonema clevei*
3. *Diatoma hiemale* v. *mesodon*
4. *Fragilaria virescens*
5. *Synedra rumpens*
6. *Cymbella sinuata*
7. *Gom. subclavatum*
8. *Cym. minuta*
9. *Achnanthes minutissima*
10. *Nitzschia paleaceae*
11. *Navicula cryptocephala* v. *veneta*
12. *Fra. vaucheriae*
13. *Cocconeis placentula* v. *euglypta*
14. *Ach. lanceolata*
15. *Gom. angustatum*
16. *Rhoicosphenia curvata*
17. *Coc. pediculus*
18. *Nav. cryptocephala*
19. *Nav. tripunctata*
20. *Nav. minima*
21. *Nit. palea*
22. *Nit. frustulum*
23. *Cyclotella meneghiniana*
24. *Nav.* sp.1
25. *Nav. seminuloides*

Asterisks indicate taxa found at only one site in the region and which comprised >1% of that site's periphyton collection. Blanks indicate taxa not found at any sites in the region.

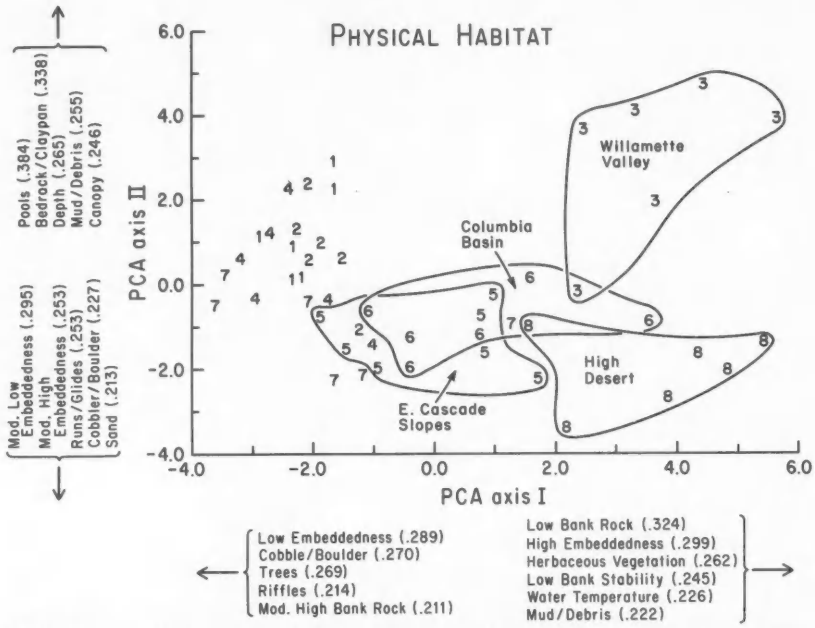

FIG. 8. Regional patterns in physical habitat measures shown by PCA ordination of 49 sites using arcsin square root transformed physical habitat data. Numbers specify ecoregions as in Fig. 1. Variables loading >0.20 are listed for each axis. Loadings are in parentheses.

ionic strength range; and the East Cascade Slopes showed the greatest spread among the montane regions.

Regional Patterns

While Omernik's map is not explicitly hierarchical, our results show a hierarchy of differences among these eight ecoregions. Although the pattern varies somewhat across the five groups of variables, the clearest, broad-scale regional differences are between the nonmontane (Willamette Valley, Columbia Basin, High Desert) and montane ecoregions. This pattern can be seen in the "species signatures" (Fig. 3, 5, 7) and in the ordinations. The montane ecoregions tend to have low first axis ordination scores and, except for the periphyton data, the nonmontane regions have high scores.

At a finer level of resolution, clear regional differences exist among the nonmontane regions. The Willamette Valley streams are consistently most different from those in all other regions. Streams rising in the Willamette Valley are relatively warm, enriched, and turbid and have high ionic strength, deep pools, and highly embedded stream bottoms with claypan and/or muddy substrates. These streams have the greatest fish species richness and diversity, and largest number of exotic species, and the fewest salmonids. The Willamette Valley periphyton assemblages are distinct from those in other regions and also have greater taxa richness and diversity. Conversely, the macroinvertebrate assemblages have low richness and diversity, are lacking several common insect families, and have the highest proportions of noninsects.

Differences between streams in the Columbia Basin and the High Desert are not as distinct. Of these two regions, the Columbia Basin tends to be more similar to the Willamette Valley as shown by Axis II ordination scores for macroinvertebrates and fish, by their high PCA scores for water quality measures, and by their similar taxa signatures for macroinvertebrates (Fig. 2, 4, 5, 9). The physical habitat of the High Desert streams is similar to that of the Willamette Valley (i.e. similar first axis scores) but is shallower, with fewer pools, and more runs, glides, cobbles, boulders, and sand. Columbia Basin streams intergrade between the High Desert character and a more montane physical habitat (Fig. 8). Conversely, the water quality of the High Desert intergrades between the montane regions and the other two regions (Fig. 9).

The East Cascade Slopes is a transitional region; its land form has elements of the high mountains and lower gradient areas (Omernik and Gallant 1986), and it is leeward of the Cascade Mountains and is therefore generally drier than the other montane regions. Four of the East Cascade Slopes sites have a generally nonmontane physical habitat (Fig. 8). Of these sites, one had a nonmontane macroinvertebrate assemblage (Fig. 4, at 215, 175), one had *Rhinichthys osculus*, and two had *Cottus perplexus*; both species are widespread and generally tolerant of a wide range of habitats (Wydoski and Whitney 1979; Lee et al. 1980). The East Cascade Slopes' water quality is definitely of the montane type but is relatively variable (large range of nutrient and ionic strength scores).

Compared with the four ecoregions discussed above, the regional differences among the remaining montane streams are subtle. Their macroinvertebrate assemblages are effectively indistinguishable at this level of resolution (Fig. 4, 5). Among the other variables, there are features that appear to distinguish these regions. The Sierra Nevada sites are nutrient poor, depauperate of fish species, and generally have more pools and bedrock and fewer runs and glides than do the other high mountain regions. The Coast Range is the only one of these regions which has a large proportion of low mountains, yet its physical habitat features are similar to the Sierra Nevada (Fig. 8; Table 5).

TABLE 5. Physical habitat measures loading >20% on PCA Axes I and II. Each site's values are the means of transect scores. Shown are regional medians with ranges in parentheses.

	Sierra Nevada	Blue Mountains	Cascades	East Cascade Slopes	Coast Range	High Desert	Columbia Basin	Willamette Valley
Water temperature (°C)	13.0 (11.5–15.5)	10.5 (8.5–12.5)	12.0 (11.0–13.0)	10.0 (8.5–14.0)	13.2 (9.0–16.0)	17.5 (13.0–20.0)	19.5 (16.0–24.0)	17.5 (15.0–22.0)
Riparian vegetation								
Trees	48 (35–100)	100 (86–100)	100 (100–100)	97 (0–100)	100 (100–100)	0 (0–55)	17 (0–91)	73 (0–89)
Herbaceous	30 (0–35)	0 (0–0)	0 (0–0)	0 (0–17)	0 (0–0)	80 (27–100)	20 (3–75)	10 (0–84)
Bank stability								
0–25%	0 (0–3)	3 (0–30)	0 (0–6)	0 (0–78)	0 (0–0)	91 (0–100)	0 (0–28)	17 (0–46)
Stream morphology								
Riffles	47 (29–69)	36 (16–50)	39 (13–45)	27 (0–30)	46 (6–57)	15 (7–90)	24 (15–42)	3 (0–32)
Runs/glides	20 (8–43)	52 (38–81)	41 (24–71)	70 (7–100)	19 (7–48)	42 (10–82)	19 (0–70)	18 (0–30)
Pools	26 (6–48)	0 (0–13)	15 (0–45)	3 (0–30)	30 (18–76)	18 (0–78)	17 (0–38)	81 (42–100)
Canopy	56 (35–78)	25 (7–57)	61 (26–77)	11 (5–96)	86 (35–99)	0 (0–28)	36 (0–79)	53 (0–71)
Bank rock content								
0–25%	0 (0–3)	15 (0–100)	0 (0–0)	70 (0–100)	0 (0–3)	100 (9–100)	24 (0–100)	100 (94–100)
50–75%	73 (0–94)	10 (0–65)	51 (4–94)	0 (0–84)	0 (0–73)	0 (0–23)	8 (0–43)	0 (0–3)
75–100%	9 (0–100)	18 (0–100)	14 (0–96)	0 (0–18)	100 (3–100)	0 (0–0)	33 (0–100)	0 (0–0)
Water depth (cm)	39 (21–45)	34 (23–39)	35 (22–48)	33 (20–71)	40 (29–49)	21 (12–37)	23 (13–46)	38 (20–85)
Embeddedness								
0–25%	93 (67–100)	62 (20–100)	92 (3–100)	23 (3–100)	62 (7–85)	2 (0–30)	7 (0–45)	2 (0–17)
25–50%	4 (0–34)	14 (0–54)	8 (0–58)	41 (0–73)	25 (0–77)	18 (6–70)	26 (0–80)	1 (0–51)
50–75%	0 (0–0)	3 (0–48)	0 (0–26)	13 (0–27)	1 (0–17)	21 (0–66)	15 (0–46)	3 (0–27)
75–100%	0 (0–30)	0 (0–24)	0 (0–13)	10 (0–22)	0 (0–47)	38 (0–63)	17 (0–94)	79 (29–100)
Stream substrates								
Mud/debris	0 (0–0)	0 (0–6)	0 (0–0)	0 (0–15)	0 (0–0)	2 (0–18)	0 (0–17)	50 (0–100)
Cobble/boulder	74 (67–81)	87 (43–100)	82 (42–96)	74 (0–94)	81 (6–100)	40 (0–57)	90 (46–100)	0 (0–0)
Bedrock/hardpan	22 (3–30)	0 (0–0)	4 (0–55)	0 (0–3)	9 (0–47)	0 (0–7)	0 (0–0)	12 (0–100)
Sand	0 (0–0)	0 (0–6)	0 (0–13)	5 (0–20)	0 (0–0)	9 (0–43)	0 (0–0)	0 (0–39)

Regionally, the Coast Range's water quality is similar to the Cascades and the East Cascade Slopes. The Coast Range fish assemblages are generally distinguished from the other montane regions by *Oncorhynchus kisutch*, *Lampetra tridentata*, and *Cottus perplexus*. The Cascades and Blue Mountains appear to be the most similar regions. Their streams generally have more runs and glides and fewer pools than the other montane regions. Their fish assemblages are similar (Fig. 3), with the Blue Mountains distinguished by *Salvelinus confluentus*. Their water quality is also similar, with the Blue Mountains having generally higher ionic strength (Fig. 9).

Other Geographic Models

Although our study was designed specifically to evaluate the correspondence between Omernik's ecoregions and spatial patterns of small stream ecosystems, an assessment of other geographic models (maps) is possible. This assessment can only be general and qualitative because (1) we cannot precisely locate

TABLE 6. Regional patterns in water quality measures. Shown are median values with ranges in parentheses.

	Sierra Nevada	Blue Mountains	Cascades	East Cascade Slopes	Coast Range	High Desert	Columbia Basin	Willamette Valley
Conductivity (μmho)	105 (54–215)	72 (57–167)	45 (33–132)	45 (31–104)	47 (41–138)	138 (70–356)	313 (281–414)	184 (155–298)
Nitrate/ nitrite (mg/L)	0.30 (0.10–1.60)	0.30 (0.20–0.70)	0.40 (0.05–5.30)	0.30 (0.20–29.30)	1.00 (0.50–2.00)	0.35 (0.20–5.30)	9.20 (0.40–30.90)	9.65 (1.40–80.30)
Kjeldahl nitrogen (mg/L)	0.25 (0.25–1.10)	0.70 (0.25–1.20)	0.62 (0.25–3.90)	0.70 (0.25–3.80)	1.20 (0.80–2.80)	1.10 (0.25–5.30)	3.15 (0.50–7.10)	4.20 (1.90–10.20)
Total phosphorus (mg/L)	0.05 (0.05–0.10)	0.15 (0.05–0.50)	0.05 (0.05–0.10)	0.30 (0.10–0.50)	0.20 (0.05–0.30)	0.65 (0.20–0.80)	1.10 (0.40–4.20)	1.70 (0.70–4.20)
Total organic carbon (mg/L)	4.8 (0.8–13.5)	1.6 (1.3–2.5)	2.8 (2.2–10.0)	1.6 (1.0–3.6)	3.3 (0.5–8.7)	6.7 (3.8–12.0)	9.1 (2.0–44.0)	5.7 (2.8–8.9)

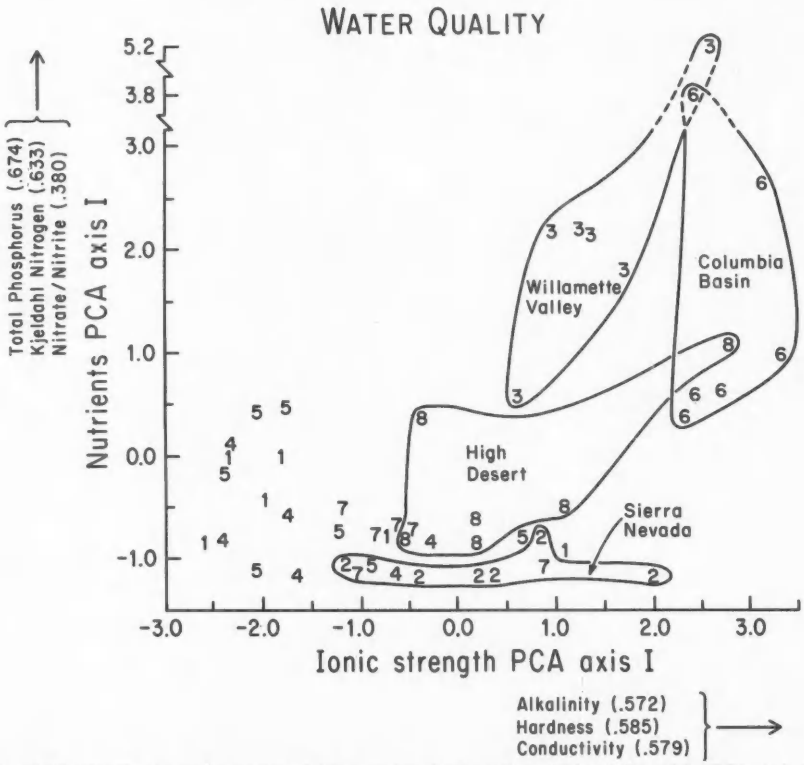

FIG. 9. Regional patterns in nutrient richness and ionic strength variables indicated by PCA Axis I scores for each. Variables and their loadings are shown for each axis.

our sampling sites on the other maps (scale 1:5,000,000 or greater); thus we cannot verify that the watersheds are entirely within a region; and (2) our fairly balanced sampling scheme could not be maintained.

We are aware of only one other explicit geographic classification (i.e. a map) for streams, that of Brussock et al. (1985). They synthesized seven stream regions in the conterminous United States primarily from patterns in runoff, gradient, and lithology to describe channel form. Their Ephemeral region encompasses the High Desert ecoregion of our study, while their Western Mountain region includes all other ecoregions in Oregon. They proposed that streams in the Ephemeral region are "in most respects independent of hypothetical stream models [RCC]." Our results for fishes, macroinvertebrates, physical habitat, and, to a lesser extent, water quality show that streams in the High Desert are markedly different from the "typical" mountain stream. However, our results also show the Willamette Valley and Columbia Basin (both within their West-

ern Mountain region) as different from mountain streams. Thus for some purposes, Brussock et al.'s (1985) regions are too coarse for Oregon and probably much of the mountain and intermountain West.

Bailey's (1976, 1981) maps of ecoregions were not specifically designed to classify streams. However, Omernik's maps were developed with an approach similar to Bailey's and with several of the same base maps (J. Omernik, U.S. Environmental Protection Agency, Corvallis, OR, pers. comm.). In addition, Bailey's maps are generally available and have been used as a geographic framework for a variety of environmental studies. Bailey's hierarchical map provides several levels of resolution; however, his "sections" are generally at the same resolution as Omernik's ecoregions.

Bailey (1976) mapped nine ecoregion sections in Oregon. Five of his regions correspond somewhat to Omernik's Willamette Valley, Blue Mountains, Sierra Nevada, East Cascade Slopes, and, to a lesser extent, Cascades ecoregions. Bailey's remaining four regions are mapped in such a way as to preclude precise evaluation with our data. Thus, if the Ponderosa Shrub Forest section, which is analogous to the East Cascade Slopes, is reclassified as montane, then Bailey's section level ecoregions may also be a useful framework for streams in Oregon.

Conclusion

Based on this study and similar studies in Arkansas (Rohm et al. 1987) and Ohio (Larsen et al. 1986; Whittier et al. 1987), we propose that Omernik's (1987) map of ecoregions of the conterminous United States can serve as a geographic framework for classifying streams. As a whole, streams within an ecoregion tend to be like other streams in that region and unlike streams in other regions. Regions with distinctly different land characteristics have streams that are more distinct; transitional regions have transitional characteristics. This scheme provides an explicit framework for the implicit knowledge that most stream ecologists already have.

Each research or management issue has its own appropriate level of spatial resolution. For example, Frissell et al. (1986) proposed a hierarchical stream classification, the top level (largest units) of which covers stream lengths on the order of 10^3 m. It seems likely that Omernik's ecoregions can be used in conjunction with a system such as Frissell et al.'s (1986) to form a more comprehensive classification. Tools such as these, which group similar streams, are of obvious value to both resource managers and researchers for stratifying natural variability.

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Evidence of Selection for Vertebral Number of Fry in Peamouth, *Mylocheilus caurinus*

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Swain, D. P. 1988. Evidence of selection for vertebral number of fry in peamouth, *Mylocheilus caurinus*. Can. J. Fish. Aquat. Sci. 45: 1279–1290.

Laboratory-reared peamouth fry (*Mylocheilus caurinus*) were exposed to predation by pumpkinseed (*Lepomis gibbosus*) or smallmouth bass (*Micropterus dolomieui*) both before and after vertebrae were developed (i.e. countable) in fry. Vertebral counts of survivors were compared with those of unexposed controls (after further laboratory rearing when experiments were conducted before vertebrae were countable). Predation was selective for vertebral number only in experiments at the larger sizes when vertebrae were well developed in fry. Selection for vertebral number did not vary between temperature (15 or 25°C) nor between cover (presence or absence) treatments and was not attributable to selection for prey length. Vertebral counts of wild fry were monitored in Holden Lake, British Columbia, between May 14 and July 1, 1984. Differences in vertebral number between age or length classes of fry in the lake that were not attributable to variation in the vertebral counts of fry entering the lake indicated selection for vertebral number of fry over a narrow range of lengths (9.3–10.6 mm). Selection favoured a low number (44) at small lengths and a high number (45) at greater lengths in this range.

Des alevins de ménés deux-barres (*Mylocheilus caurinus*) ont été exposés à la prédation par le crapet-soleil (*Lepomis gibbosus*) ou l'achigan à petite bouche (*Micropterus dolomieui*) avant et après le développement de vertèbres apparentes. Le nombre de vertèbres des survivants a été comparé à celui de poissons témoins non exposés (après la poursuite de l'élevage en laboratoire dans les cas où les essais avaient été réalisés avant que les vertèbres ne puissent être dénombrées). La prédation n'était sélective quant au nombre de vertèbres que dans les essais portant sur les alevins plus gros dont les vertèbres étaient bien développées. La sélection visant le nombre de vertèbres n'a pas varié en fonction de la température (15 ou 25°C) ou du couvert (présence ou absence) et ne pouvait être attribuée à une sélection portant sur la longueur des proies. Des dénombrements de vertèbres d'alevins sauvages ont été réalisés au lac Holden (Colombie-Britannique) du 14 mai au 1er juillet 1984. Les écarts du nombre de vertèbres entre les classes d'âges ou de longueurs des alevins présents dans le lac qui ne pouvaient être attribués à la variation du nombre de vertèbres des alevins pénétrant dans le lac indiquaient une sélection quant au nombre de vertèbres portant sur une gamme étroite de longueurs (9,3–10,6 mm). La sélection favorisait un nombre faible (44) de vertèbres aux petites tailles et un nombre élevé (45) aux tailles plus grandes de cette gamme.

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Although meristic characters such as the number of vertebrae have long been used to delineate stocks, races, and species of fish (Tester 1949; Lindsey 1961; Ni 1982; Meng and Stocker 1984), little is known of the adaptive significance of variation in these characters. Surprisingly wide variation in these characters often occurs within single fish populations. This variation is partly due to environmental influences, notably an effect of temperature on the number of parts formed in embryos (Lindsey and Arnason 1981), but it also has a genetic component (Lindsey 1962; Ali and Lindsey 1974; Kirpichnikov 1981; Tave 1984; Leary et al. 1985). This genetic variation may persist within populations because, although selection may act against extreme numbers of parts, the precise number of parts may be without selective significance over a moderate range of intermediate numbers (Fowler 1970).

Precise vertebral number has, however, recently been shown to be of functional significance in the threespine stickleback (*Gasterosteus aculeatus*) (Swain and Lindsey 1984; Swain 1986). Over a narrow range of lengths, both the swimming performance of fry and their ability to escape predation depended on vertebral number. Widespread trends in vertebral number among populations of related fishes (e.g. Jordan's rule, Jordan 1892; pleomerism, Lindsey 1975) suggest that this selective significance demonstrated in *G. aculeatus* may be general in fishes. To test this possibility, I have looked for evidence of selection for vertebral number of fry in an unrelated fish species, the peamouth *Mylocheilus caurinus* (Family Cyprinidae). I present here a demonstration of selective predation for vertebral number of peamouth fry in the laboratory and evidence of selection for vertebral number of fry in the wild.

For studies of selection in the wild, this species has the advantage over *G. aculeatus* that spawning occurs in discrete

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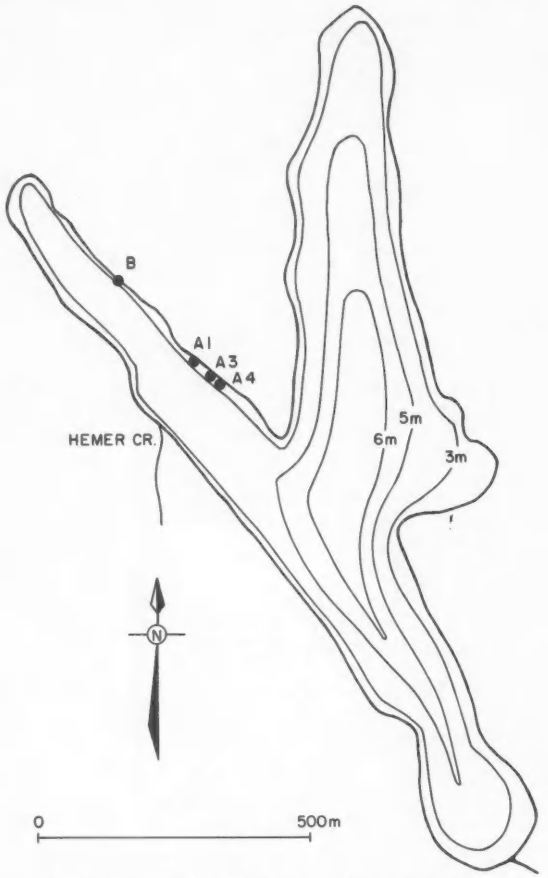

FIG. 1. Map of Holden Lake, B.C., showing sampling sites.

pulses and, in the population studied, is confined to a discrete area (an inlet stream). Thus, it was possible both to monitor the vertebral counts of recently hatched fry entering the population and to follow changes in vertebral count with age or length within a discrete cohort of fry.

Mylocheilus also differs from *Gasterosteus* in the timing of vertebral development. In *Gasterosteus*, vertebrae are already well developed (i.e. countable) when fry become free-swimming and are first exposed to selection for swimming performance (Swain 1986). In *Mylocheilus*, fry become free-swimming before vertebrae are well developed. Thus, in this study, it was possible to examine selection for vertebral number both before and after vertebrae were developed, providing information on whether the structures directly involved in selection are the vertebrae themselves, or some related feature of the axial segmentation such as myomeres or nerves.

Material and Methods

Study Area

Peamouth were collected from Holden Lake (49°06′N, 123°50′W), a small eutrophic lake near Nanaimo, Vancouver Island, and from its main inlet stream, Hemer Creek (Fig. 1). Although peamouth may have been introduced by man to some areas of Vancouver Island, they are probably native to this lake (Clark and McInerney 1974). The lake has a surface area of 40 ha and maximum depth of 6 m. Potential predators of fry in the lake include adult peamouth, threespine stickleback, pumpkinseed (*Lepomis gibbosus*), sculpins (*Cottus* sp.), trout (*Salmo clarki*), and a variety of macroinvertebrates, notably dragonfly nymphs.

Predation Experiments

Ripe adults were collected from Holden Lake on April 28 and May 11, 1983, and 7 or 27 pairs respectively were artificially crossed in the laboratory. Embryos from each set of crosses (designated PM1 and PM8, respectively) were incubated in separate 20-L tanks at 14°C. After hatching, fry were fed *Artemia* nauplii twice daily, with occasional supplements of nematodes and natural plankton. Conditions were crowded and growth and development slow compared with those of fry in the wild.

For each of five experiments, offspring of a single collection of parents were distributed in lots of 10–30 fish alternatively among six 190-L green fiberglass tanks (110 × 50 × 34 cm) 22–56 d after hatching. Two control tanks received about 200 (experiments 1–4) or 110 (experiment 5) fish each, and four experimental tanks received about 500 (experiments 1–4) or 275 (experiment 5) fish each. One control and two experimental tanks were maintained at 15°C and the remaining tanks at 25°C. Cover (shredded plastic) was suspended in one experimental tank at each temperature. Tanks were illuminated by overhead fluorescent lights on a photoperiod of 16.5 h light : 7.5 h dark. Water flow was 1–2 L/min in each tank. After about 15 h of acclimation, fry in experimental tanks were exposed to predation by pumpkinseed or smallmouth bass (*Micropterus dolomieui*) until 50–65% of the initial number had been eaten. Predators were usually introduced in groups of 10 or less at intervals of 1 h or more, to a maximum of 10–40 bass or 10 pumpkinseed per tank. Fry were fed *Artemia* nauplii twice daily during experiments. Details are given in Table 1.

In all but experiment 5, some fry were below the length at which vertebrae are sufficiently developed to be countable. Vertebrae were considered "developed" when the positions of all intervertebral sutures were clearly indicated by thickenings of the notochord and by variation in staining. Vertebrae were "undeveloped" in 100, 58, 26, and 14% of fry at the end of experiments 1–4, respectively. At the end of each of these experiments, the fry in each tank were divided into quarters using a "plankton splitter." One quarter of the fry were preserved for length measurements (hereafter "nonreared" fry) and the remainder reared to a larger size (hereafter "reared"fry). All fry had developed vertebrae and were preserved at the end of experiment 5.

Postexperimental rearing was at about 17°C in 4-L plastic jars or 10-L aquaria. Water was standing but aerated. Fry were fed *Artemia* nauplii twice daily. Debris was siphoned from containers, mortalities recorded, and fresh water added every few days.

Vertebral counts reported for experiments 1 and 2 are entirely of reared fry because vertebrae were undeveloped in all or most nonreared fry at the end of these experiments. Counts reported for experiments 3 and 4 are of both reared and nonreared fry; most nonreared fry had developed vertebrae in these experiments, and counts of reared and nonreared fry differed by less than 1% over all treatments in both experiments. Vertebral

TABLE 1. Description of predation experiments. Predators are pumpkinseed (A) or smallmouth bass (B). Predator-hours (pred-h) are calculated using lighted periods only. Cover present (Y) or absent (N). Par. = parental crosses; Tmt. = predation treatment: control (con.) or experimental (exp.); Pres. = preserved at end of experiment without further rearing.

					Duration		Predators					No. surviving			Length (mm) of preserved survivors	
							Length (mm)			%	No. eaten per			Rearing mortality		
Exp.	Par.	Tmt.	Temp. (°C)	Cover	h	Pred-h	Type	Mean	SD	eaten	pred-h	Pres.	Reared	(%)	Mean	SD
1	PM8	Con.	15	N	28	0	—	—	—	0	—	60	155	5.8	8.84	0.37
			25	N	27	0	—	—	—	0	—	49	161	23.0	9.02	0.40
		Exp.	15	Y	26	203	A	31.2	2.6	55	1.35	70	155	10.3	8.95	0.32
			15	N	22	154	A	30.4	2.1	52	1.71	62	186	37.1	8.86	0.30
			25	Y	23	140	A	29.4	2.4	61	2.34	37	168	19.6	9.05	0.29
			25	N	24	148	A	30.0	2.6	66	2.42	54	131	12.2	9.06	0.33
2	PM1	Con.	15	N	78	0	—	—	—	0	—	53	149	20.8	9.89	0.38
			25	N	54	0	—	—	—	0	—	53	149	0.0	10.13	0.58
		Exp.	15	Y	98	2208	B	14.8	1.7	48	0.12	75	201	1.0	10.03	0.49
			15	N	96	2082	B	14.5	2.1	51	0.13	61	196	17.9	9.85	0.55
			25	Y	52	830	B	16.7	2.3	64	0.40	34	153	0.0	10.23	0.57
			25	N	54	868	B	16.6	2.1	64	0.38	41	146	0.0	10.18	0.59
3	PM8	Con.	15	N	49	0	—	—	—	0	—	58	142	7.0	10.14	0.53
			25	N	29	0	—	—	—	0	—	48	152	5.3	10.38	0.47
		Exp.	15	Y	48	614	B	22.8	4.3	61	0.50	52	142	3.5	10.42	0.48
			15	N	46	589	B	21.8	4.0	68	0.58	45	116	15.5	10.67	0.48
			25	Y	28	192	B	23.8	4.8	60	1.56	57	143	1.4	10.56	0.46
			25	N	30	214	B	23.3	5.5	57	1.33	50	163	7.0	10.47	0.41
4	PM1	Con.	15	N	73	0	—	—	—	0	—	48	215	0.0	10.81	0.74
			25	N	49	0	—	—	—	0	—	43	162	0.0	10.86	0.67
		Exp.	15	Y	70	1599	B	17.8	2.8	49	0.15	86	167	0.0	10.77	0.71
			15	N	74	1751	B	17.5	2.0	49	0.14	74	187	0.0	10.91	0.74
			25	Y	47	610	B	19.0	0.8	55	0.44	54	167	0.0	11.12	0.72
			25	N	48	623	B	19.0	2.8	57	0.46	47	163	0.0	11.14	0.85
5	PM8	Con.	15	N	31	0	—	—	—	0	—	109	0	—	11.60	0.62
			25	N	27	0	—	—	—	0	—	110	0	—	11.84	0.73
		Exp.	15	Y	30	377	B	23.5	4.8	58	0.43	114	0	—	12.25	0.89
			15	N	30	359	B	23.6	5.2	58	0.44	116	0	—	12.11	0.85
			25	Y	26	150	B	25.7	5.5	60	1.10	111	0	—	12.19	0.81
			25	N	26	152	B	25.7	6.0	60	1.09	110	0	—	12.26	0.82

counts reported for experiment 5 are entirely of fry without postexperimental rearing.

In experiments 3 and 5, vertebral counts are compared between predation treatments among fish above or below a specified length at the end of experiments. In experiment 3, reared fry are assigned to the small or large length class based on the percentages of nonreared fry in each length class.

Selection in Wild Fry

Spawning appeared to be confined to Hemer Creek and occurred on several nights between late April and early June in 1984. After hatching, fry migrated into the lake at night, beginning at dusk. In 1984, the first fry migration into the lake occurred on the evening of May 12 or 13. Fry migrating into the lake were collected between May 14 and July 5, using a Surber sampler set about 15 m upstream of the lake. Sets were of 5- or rarely 15-min duration between 2200 and 2300 and sampled the entire water column. Additional sets were made at earlier or later times on two occasions. Collections were made every day or two. Fry were also collected from the lake between May 14 and June 27 at sites A1, A3, and A4 and between May 31 and June 27 at site B (Fig. 1). Lake collections were made by dip net (25 × 17 cm gape) from a canoe floating near shore.

Vertebrae were undeveloped in fry collected from the creek, in most fry collected from the lake in May, and in smaller fry collected from the lake in June. Fry collections from the creek were divided into halves or quarters using a plankton splitter. One half or one quarter were reared in the laboratory to a larger size and the remainder killed in anaesthetic (2-phenoxyethanol) and preserved in 10% buffered formalin. Fry collections from the lake were treated in one of three ways:

(1) All fry collected on May 14 and 18 were reared to a larger size because sample sizes were relatively small.

(2) Fry collected between May 20 and June 18 and on June 27 were divided into two groups using a plankton splitter. One group was killed and preserved. Before June 11 or 14 (depending on collection site), the second group was reared to a larger

TABLE 2. Effect of predation on mean length of fry. E–C is the difference in mean length between experimental and control groups. Means are the unweighted averages of the means within temperature treatments. E–C is also shown within temperature treatments when the interaction (INT) between predation (PT) and temperature (TMP) treatments is significant. *Variances heterogeneous; Brown–Forsythe test used.

			Probability		
Exp.	Temp.	E–C	PT	TMP	INT
1	Both	0.05	0.26*	0.0001	0.72
2	Both	0.07	0.31	0.0001*	0.88
3	Both	0.27	<0.0001	0.052	0.026
	15°C	0.40	<0.0001		
	25°C	0.14	0.081		
4	Both	0.14	0.11	0.061	0.17
5	Both	0.48	<0.0001*	0.028	0.11

size. After these dates, fry in the second group were anaesthetised and divided into size groups. Fry in the smaller groups were reared to a larger size and those in the larger group preserved.

(3) Fry collected on June 22 were anaesthetised and divided into small (<9.5 mm) or large (>9.5 mm) size classes. Those in the small size class were reared to a larger size and those in the large class preserved.

Laboratory rearing was at about 14–18°C in nylon mesh baskets (16 × 12 × 13 cm) suspended in Plexiglas tanks (75 × 60 × 30 cm). Water was flowing at about 1 L/min. Fry were fed *Artemia* nauplii twice daily and mortalities recorded daily.

Measurements and Counting Methods

Fry were killed in anaesthetic, preserved in 10% buffered formalin, stained with alcian blue and alizarin red, cleared with trypsin and 0.5% KOH, and stored in glycerin (following Dingerkus and Uhler (1977), except that 2 mg alcian blue/mL of stain solution was used here). Standard length was measured using a binocular microscope fitted with an ocular micrometer. Counts were made of centra, excluding the urostyle and including members of the Weberian complex. The last or second last vertebra frequently bore two neural and occasionally two haemal arches. Such complex vertebrae were counted as one. Counts were equivocal, and were excluded from analyses, in less than 1.5% of fish with developed vertebrae due to other vertebral irregularities.

Fry preserved upon collection from the creek were not cleared and stained, but were measured. Lengths of these fry cannot be compared directly with those of cleared and stained fry. Clearing and staining appeared to reduce fry length by about 0.5–0.7 mm (about 6–8%).

Statistical Computations

Lengths were compared by ANOVA, calculated using BMDP7D (Dixon 1981). Variances were tested for heterogeneity using Levene's test (Brown and Forsythe 1974a) and means compared using the Brown–Forsythe test (Brown and Forsythe 1974b) when variances were significantly ($p < 0.05$) heterogeneous. Linear regressions were calculated using BMDP1R and equality of slopes tested using BMDP1V. Count distributions were compared by chi-squared tests, calculated using BMDP4F. To avoid classes with small expected values, counts were grouped as 44, 45, and "other," or as 44 and "other" when sample sizes were small (see Table 5). Yates correction for continuity was used in 2 × 2 comparisons with sample sizes under 200.

Results

Predation Experiments

The mean length of survivors of predation in experimental groups tended to be slightly greater than that of fry in control groups at the end of all experiments (Table 1). Because fry densities were greater in experimental than in control tanks, and

TABLE 3. Regressions between vertebral number and length among fry exposed (experimental) or unexposed (control) to predation. Fry were either preserved at the end of experiments or reared to a larger size.

Exp.	Type	Predation treatment	*N*	Intercept	Slope	Probability of zero slope
1	Reared	Control	266	48.8435	0.0405	0.13
		Experimental	494	44.3808	−0.0015	0.93
2	Reared	Control	258	44.8603	−0.0439	0.73
		Experimental	634	44.3083	0.0015	0.92
3	Preserved	Control	60	42.1349	0.2083	0.24
		Experimental	170	45.4714	−0.0953	0.41
	Reared	Control	268	43.1562	0.1147	0.002
		Experimental	500	43.9449	0.0398	0.20
4	Preserved	Control	82	45.1025	−0.0708	0.41
		Experimental	216	43.9354	0.0330	0.48
	Reared	Control	358	45.0395	−0.0605	0.035
		Experimental	675	44.5598	−0.0217	0.27
5	Preserved	Control	219	44.2292	0.0194	0 72
		Experimental	451	43.6556	0.0635	0.043

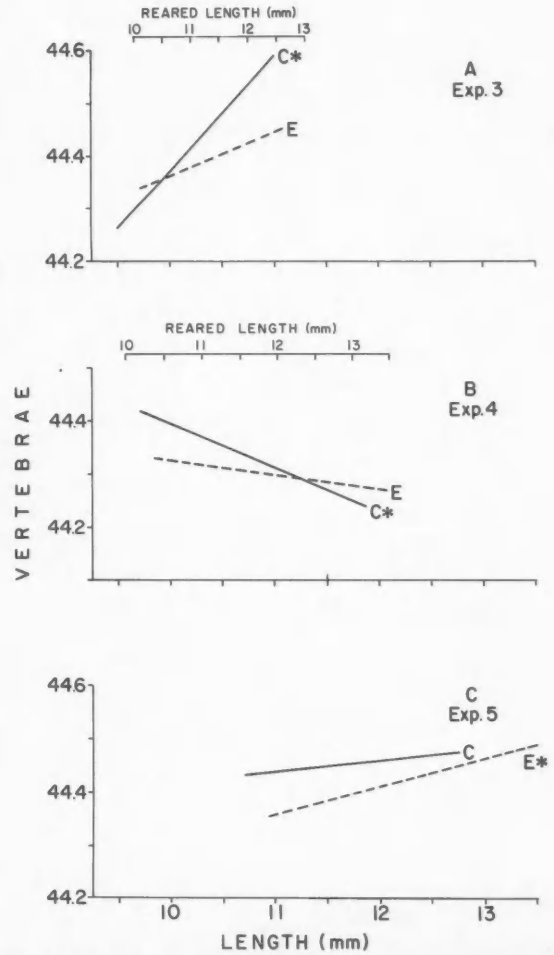

FIG. 2. Regressions between vertebral number and length in control (C) and experimental (E) groups, experiments 3–5. Length shown on lower axis is at the end of experiments (for experiments 3 and 4, regressions are among fish with postexperimental rearing, but lines are transposed to the estimated lengths before rearing; scales of reared lengths are also shown). Asterisks indicate slopes significantly different from zero ($p < 0.05$). Lines drawn over the range $\bar{X} \pm 1.5$ SD.

Artemia rations were adjusted for approximate fry numbers in each tank, these differences in length presumably reflect size-selective predation rather than differences in growth between the two groups. However, size selection was statistically significant only in experiments 3 and 5 (Table 2). Rates of predation in these two experiments were intermediate to those in the experiments without significant size selection (Table 1).

Differences between control and experimental groups in the regression of vertebral number on length provide circumstantial evidence of selective predation for vertebral number in some experiments (Table 3). Regressions are shown grouped over replications within predation treatments because slopes did not differ among such replications in any experiment ($p > 0.18$). In experiments 1 and 2, slopes differed significantly from zero neither in control nor in experimental groups. Among fry with postexperimental rearing in experiments 3 and 4, slopes differed significantly from zero in control but not in experimental groups. In experiment 5, slope differed significantly from zero in the experimental but not in the control group. Differences in significance between control and experimental groups might be explained by the difference in sample size between the two groups in experiment 5, but not in experiments 3 and 4. In the latter experiments, regressions were significant in the group with the smaller sample size. In these latter experiments, regressions were also significant ($p < 0.05$) within replications of control groups but not within those of experimental groups. Differences in the significance of regressions between control and experimental groups could result from selective predation for vertebral number. The observed changes in significance between groups exposed or unexposed to predation are consistent with selection favouring fry with 44 vertebrae at larger sizes in the range 9.2–11.2 mm ($\bar{X} \pm 2$ SD) in experiment 3 and at smaller sizes in the ranges 9.4–12.3 mm in experiment 4 and 10.4–13.1 mm in experiment 5 (Fig. 2).

TABLE 4. Vertebral counts of fry exposed (experimental) or unexposed (control) to predation. p is the probability that count distributions do not differ between predation treatments.

Exp.	Predation treatment	Vertebral number (%) ≤43	44	45	≥46	N	p
1	Control	4.1	59.9	33.3	2.6	267	0.065
	Experimental	2.4	60.4	36.4	0.8	503	
2	Control	0.7	64.4	34.5	0.4	278	0.19
	Experimental	2.9	61.6	35.3	0.2	654	
3	Control	1.5	57.7	38.7	2.1	333	0.31
	Experimental	2.9	55.8	38.4	2.9	683	
4	Control	2.7	62.3	34.1	0.9	440	0.18
	Experimental	1.8	67.2	30.1	0.9	891	
5	Control	0.9	54.3	42.9	1.8	219	0.76
	Experimental	1.6	55.9	40.6	2.0	451	

Vertebral counts did not differ significantly between levels of the temperature or cover treatments in experimental groups in any experiment ($p > 0.13$), nor did the nonsignificant variations in count between levels show any pattern consistent over experiments. Results are therefore grouped over temperature and cover treatments in the following analyses.

Vertebral counts of fry surviving exposure to predation did not differ significantly from those of unexposed controls in any experiment (Table 4). In experiment 1, a decrease in the frequency of fry with extreme vertebral counts after exposure to predation approached significance ($p = 0.065$). The greatest change in frequency seen in the five experiments was in increase in the frequency of fry with 44 vertebrae after exposure to predation in experiment 4 (by about 5%, $p = 0.18$). In experiments 3 and 5, selection in favour of fry with 44 vertebrae could have been obscured by size selection. Confounding with an effect of size selection can be partly avoided by comparing counts between predation treatments within restricted size classes. This procedure fails to reveal significant differences in vertebral count between survivors of predation and unexposed controls in experiment 3 (Table 5). However, in experiment 5, fry with 44 vertebrae *are* significantly more common after than before exposure to predation at lengths under 11.5 mm ($p =$

TABLE 5. Vertebral counts of fry exposed or unexposed to predation at small or large lengths in experiments 3 and 5.

Exp.	Length class (mm)	Mean length (mm)	Predation treatment	Vertebral number (%) ≤43	44	45	≥46	*N*	*p*
3	≤10.35	9.94	Control	1.7	64.4	33.3	0.6	174	0.10
		10.06	Experimental	4.2	56.1	36.9	2.8	214	
	>10.35	10.76	Control	1.3	50.0	44.9	3.8	158	0.23
		10.80	Experimental	2.4	55.5	39.4	2.8	465	
5	≤11.5	11.13	Control	1.0	53.6	43.3	2.1	97	0.031
		11.16	Experimental	—	69.9	29.0	1.1	93	
	>11.5	12.18	Control	0.8	54.9	42.6	1.6	122	0.61
		12.47	Experimental	2.0	52.2	43.6	2.2	358	

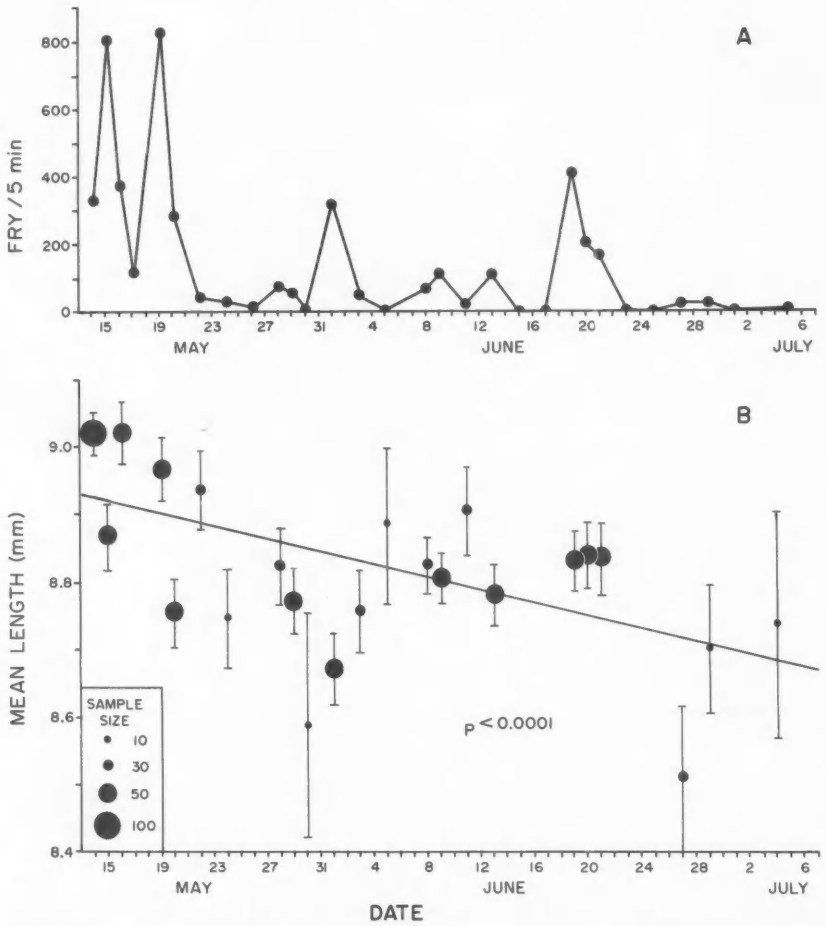

FIG. 3. Migration of peamouth fry in Hemer Creek, 2200–2300, May 14 to July 5, 1984. (A) Number of fry captured per 5-min Surber sampler set. (B) Mean lengths of migrating fry. Vertical bars are standard errors. Regression line between length and collection day (calculated on the ungrouped data) is shown. *p* is the probability that the slope of this line is zero.

0.03), but not at greater lengths (Table 5). This difference at the shorter lengths cannot be attributed to size selection.

Selection in Wild Fry

Fry migration into the lake was greatest before May 21 and usually slight thereafter (Fig. 3A). Peak migrations occurred on the evenings of May 15 and 19. Lesser peaks occurred on June 1 and 19. About 95% of fry entering the lake between 2200 and 2300 had 44 or 45 vertebrae (Table 6). Vertebral counts differed markedly between fry entering the lake before

TABLE 6. Vertebral counts of peamouth fry collected from Hemer Creek between 2200 and 2300 in May and June 1984.

Date	Rearing mortality (%)	Vertebral count (%)				No. counted
		≤43	44	45	46	
May 14	12.1	1.4	46.1	50.1	2.4	423
15	21.3	1.8	46.2	49.9	2.0	941
16	15.5	4.3	44.3	49.3	2.1	140
19	10.7	2.2	71.7	25.6	0.6	180
28	2.0	6.3	70.8	22.9	0.0	48
June 1	9.6	4.7	80.0	15.3	0.0	170
9	0.0	6.9	58.6	34.5	0.0	58
13	10.2	5.4	73.0	21.3	0.0	296
19	2.4	7.9	78.7	13.4	0.0	202

or after May 17 ($p < 0.0001$). Counts of 44 vertebrae occurred in only about 45% of fry entering before this date, but in over 70% of those entering thereafter. Average length of fry entering the lake also varied over the migration period, decreasing from early to late migration dates (Fig. 3B; $p < 0.0001$ according to linear regression analysis of the ungrouped data). Within an evening, fry migrations were apparently homogeneous over time with respect to vertebral number, but not with respect to length (Table 7). Hence, vertebral counts of fry entering the lake between 2200 and 2300 on a given evening (Table 6) are apparently representative of those entering the lake over the entire evening.

Vertebrae were undeveloped in most fry collected from the lake in May (Fig. 4). Vertebral counts produced in these fry after further rearing in the laboratory differed neither among A sites ($p = 0.85$) nor between sites A and B ($p = 0.59$). Fry are therefore grouped over collection sites in subsequent analyses. Vertebral counts of fry in the lake did differ among collection dates in May ($p < 0.0001$, Table 8). Fry with 44 vertebrae were more common on May 21 than on earlier ($p < 0.0001$) or later ($p = 0.013$) dates in May. The increase in frequency of fry with 44 vertebrae between May 18 and 21 in the lake presumably results from the increased frequency of this vertebral count among fry migrating into the lake on May 19 (Table 6). The decreased frequency of this vertebral count between fry collected from the lake on May 21 and 23 might reflect greater mortality among newly arrived fry in the lake. At any rate, vertebral count distributions were relatively homogeneous among collections made after May 21. In particular, the frequency of fry with 44 vertebrae varied little over the period May 23–31 ($p = 0.83$).

In the following analyses, three "cohorts" of fry are distinguished. Fry entering the lake before May 22 comprise cohort A; those entering the lake about June 1, cohort B; and those entering June 19–21, cohort C. An attempt to delimit cohort A is shown in Fig. 4 in which the broken line connects the approximate median lengths of fry of this cohort (estimated by eye) in each collection from the lake and the solid line shows the assumed lower size limit of this cohort in each collection. This lower limit is obtained by assuming that fry of the smallest and median lengths have similar growth rates. If smaller fry had slower growth rates, this line will have been drawn conservatively high and exclude a small proportion of fry actually in cohort A. However, this conservative procedure should also ensure that those fry assigned to cohort A do not include a significant proportion of fry entering the lake after May 21. No attempt is made to delimit cohorts B and C in Fig. 4. Cohort B can be first distinguished as the mode at small sizes in the June 4 sample and cohort C as that in the June 27 sample. Cohort C was also present in the June 22 sample, but is not shown in the figure on this date because most fry below 9 mm in length in this sample were reared to a larger size after collection (see Material and Methods).

A positive correlation between vertebral number and length might be expected in cohort A because fry entering the lake late in the period May 14–21 had fewer vertebrae than did those entering early in this period. However, such an association was not seen in any collection between May 23 and 31 (Table 9). The absence of such an association can be explained by the apparent lack of growth among fry in the lake before May 24 (Fig. 4).

On the other hand, vertebral number and length *were* associated among fry collected from the lake in June (Fig. 5). In most June samples, fry with 44 vertebrae tended to decrease in frequency as length increased. In samples collected after June 8, this tendency could be partly due to confounding between length and cohort, since fry with 44 vertebrae were more common in cohorts B and C, which entered the lake late in the season, than in cohort A, which entered early (Table 6). However, a decrease in the frequency of fry with 44 vertebrae persists between mean lengths of 9.66 and 10.25 mm when fry collected between June 4 and 22 are grouped together (bottom panel, Fig. 5). In this comparison, length and cohort membership are not strongly confounded: cohort C has been excluded from both length classes by omitting the June 27 sample, and the proportion of fry in cohort A or B fluctuates only moderately between the two length classes. Finally, this same relationship between vertebral number and fry length is seen in the June samples of cohort A alone (Fig. 6): fry with 44 vertebrae again decreased in frequency, and those with 45 vertebrae increased in frequency, between the 9.3–9.9 and 9.9–10.6 mm length classes ($p = 0.022$). Because an initial correlation between vertebral number and length has been ruled out for cohort A (Table 9), this change in frequency indicates selection

TABLE 7. Vertebral counts and lengths of peamouth fry collected from Hemer Creek at different times on the same night. $p(l)$ and $p(v)$ are the probabilities that lengths and counts, respectively, do not differ among collection times.

Date	Time	Length (mm)				No. per 5 min	Rearing mortality (%)	Vertebral number (%)				No. counted	
		Mean	SD	N	$p(l)$			43	44	45	46		$p(v)$
May 15	2215–2230	8.87	0.33	48	0.0064	807	21.3	1.8	46.2	49.9	2.0	941	0.81
16	0115–0130	9.05	0.29	48		58	27.3	5.6	44.2	50.0	0.0	54	
May 16	2115–2120	9.15	0.21	11		59	16.7	5.0	47.5	47.5	0.0	40	
	2145–2150	9.02	0.27	48	0.39	338	18.3	2.8	51.1	45.4	0.7	141	0.72
	2215–2200	9.02	0.31	48		378	15.5	4.3	44.3	49.3	2.1	142	

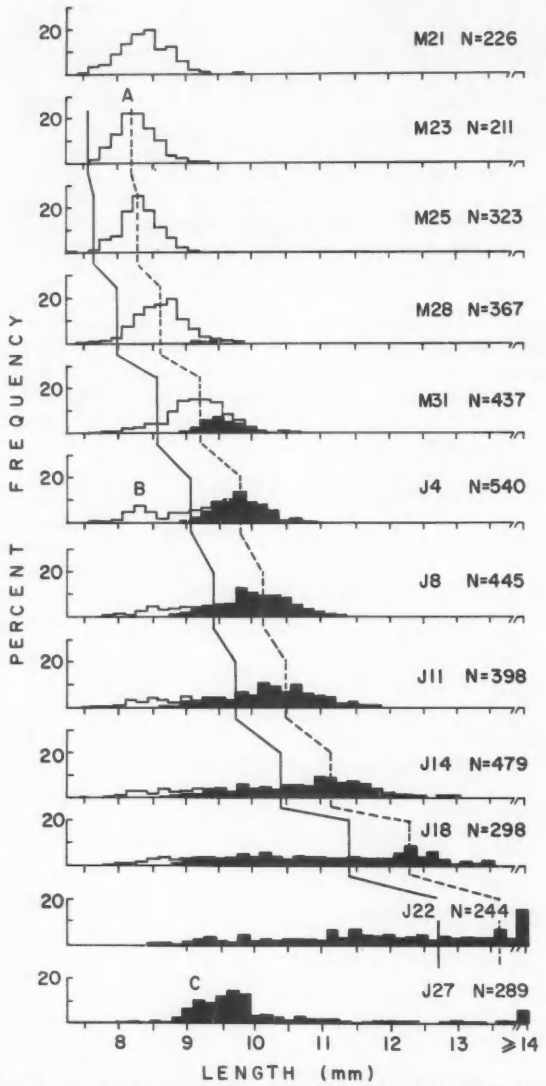

FIG. 4. Length distributions of peamouth fry collected from Holden Lake between May (M) 21 and June (J) 27, 1984. Open bars indicate fry with vertebrae undeveloped; black bars indicate those with vertebrae developed. Three cohorts are marked (A, B, C). The broken and solid lines connect the estimated median and smallest lengths, respectively, of fry in cohort A (see text). Only fry over about 9 mm in length are shown for June 22.

favouring fry with 45 vertebrae at the larger size. A similar (although nonsignificant) result can be seen comparing only the larger fry in the May 31 and June 4 samples (Table 10). Conversely, fry with 44 vertebrae were more frequent, and those with 45 vertebrae less frequent, in June samples of cohort A at lengths of 9.3–9.9 mm than in the May sample of this cohort (Fig. 6). This comparison is valid because vertebral number and length were unrelated in May samples and suggests selection favouring fry with 44 vertebrae at lengths below 9.9 mm. This result is confirmed comparing counts of smaller fry in the May 31 and June 4 samples (Table 10). Finally, vertebral counts

TABLE 8. Vertebral counts of peamouth fry collected from Holden Lake between May 14 and 31, 1984. Fry were reared to a countable size in the laboratory.

Date	Rearing mortality (%)	Vertebral count (%) ≤43	44	45	46	No. counted
May 14	16.0	3.0	50.9	44.2	1.8	165
18	24.3	3.1	43.0	53.4	0.4	223
21	30.0	3.5	68.2	27.5	0.9	349
23	7.8	6.0	60.3	32.8	0.9	232
25	9.6	1.0	61.1	37.6	0.3	306
28	10.7	2.5	62.6	33.2	1.6	364
31	11.8	2.8	59.6	36.8	0.8	497

TABLE 9. Vertebral counts of small or large fry in cohort A in samples collected between May 23 and 31.

Date	Length class (mm) After rearing	Before rearing (estimated)	Vertebral count (%) ≤43	44	45	46	N	p
May 23	≤10.6	≤8.4	7.6	60.6	31.8	0.0	132	0.86
	>10.6	>8.4	4.0	60.0	34.0	2.0	100	
May 25	9.6–10.9	7.8–8.4	1.9	59.6	37.9	0.6	161	0.17
	>10.9	>8.4	0.0	61.7	38.3	0.0	141	
May 28	9.6–10.6	8.1–8.7	2.9	64.5	30.2	2.3	172	0.41
	>10.6	>8.7	2.2	61.0	35.7	1.1	182	
May 31	10.1–10.9	8.6–9.3	4.0	55.6	39.9	0.5	198	0.55
	>10.9	>9.3	2.0	60.2	36.6	1.2	246	

of fry in cohort A vary little with length between mean lengths of 10.25 and 13.75 mm ($p = 0.87$), suggesting that selection favours neither fry with 44 nor those with 45 vertebrae at these greater lengths (over 10.6 mm).

Rearing Mortality and Other Extraneous Sources of Variation

Distinction must be made here between the size at which the ultimate vertebral number is countable and the earlier size at which the ultimate number is embryonically fixed. Ultimate vertebral number is determined by the time the last somites differentiate, well before hatching in most fishes (Fowler 1970; Lindsey 1988). In peamouth, the caudalmost axial elements (the hypurals) are the first to ossify and were visible in the smallest fry used in this study, confirming that the ultimate vertebral number was presumably already fixed in these fry. Thus, the number of vertebrae ultimately produced in individual fish could not have been altered by the environment during laboratory rearing after collection from the wild or use in predation experiments.

The mean number of vertebral produced in a group of fish may, however, have been altered by selective mortality during laboratory rearing. Rearing mortality was apparently largely due to infection by fungi, bacteria, and parasitic flukes and is not expected to be strongly selective for vertebral number at the stages of development reared here (Lindsey 1962). Vertebral counts of fry dying during postexperimental rearing were obtained for two groups in predation experiment 2. Counts of these fry (44–52.5 and 45–45.0%, $N = 40$) did not differ significantly ($p = 0.38$, grouping counts as 44 or other) from

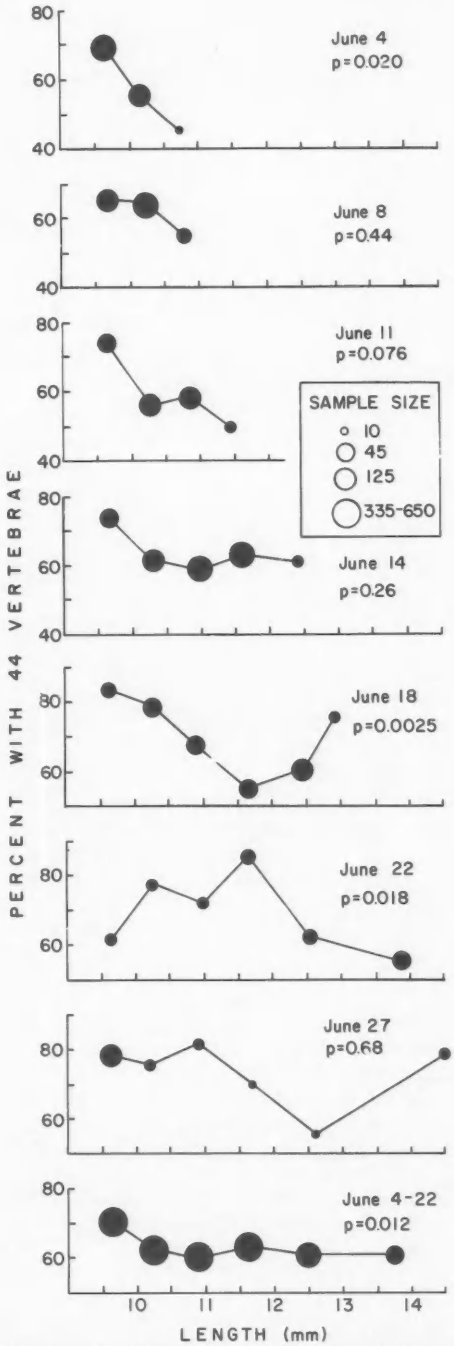

FIG. 5. Frequencies of fry with 44 vertebrae in length classes of collections between June 4 and 27. Fry are those preserved without laboratory rearing. Fry below 9.3 mm in length are omitted, since a high proportion of these smaller fry had undeveloped vertebrae. Probabilities are from chi-squared tests with counts grouped as 44 or other.

those of survivors of postexperimental rearing in these groups (44–59.9 and 45–37.8%, $N = 267$). However, sample size was small for fish that died during rearing. If rearing mortality was selective, the data suggest that it was lower for fish with 44 vertebrae. If this were so, fish with 44 vertebrae should be more frequent among survivors in replications with high mortality than in those with low mortality. This relationship was not seen in either predation experiment 1 or 2, comparing control groups with high (>20%) or low (0–6%) rearing mortality.

Results in the second part of this study, on selection in the wild, also show no indication of selective rearing mortality. No consistent pattern is seen between vertebral number and extent of rearing mortality in Tables 6 and 8. Furthermore, Swain (1986) compared replications with widely varying rearing mortality for four groups (replications were subsamples from the same Surber sampler set, or samples collected from different A sites on the same date). In no group did vertebral counts differ significantly among replications ($p > 0.24$), nor was there any consistent pattern among replications between vertebral number and the extent of rearing mortality. Thus, it is unlikely that the significant differences seen above among reared groups, or between reared and nonreared groups, result from varying extents of rearing mortality.

A difference in the rate of vertebral development between fish with different vertebral numbers is a second possible source of spurious differences among groups because vertebrae were undeveloped in some of the smaller nonreared fry but in few of the reared or larger nonreared fry. However, this difference can also be discounted as a significant cause of differences in vertebral number among groups. In the predation experiments, vertebral number and degree of development were apparently unrelated: reared and nonreared subsamples, differing by 12–24% in the proportion of fry with undeveloped vertebrae, differed by less than 1% in vertebral count frequencies. Furthermore, in the study of selection in the wild, differences between groups in the proportion of fry with countable vertebrae were small (4%) compared with differences in vertebral count frequencies (10%).

Discussion

The predation experiments described here provide some evidence of selection in favour of fry with 44 vertebrae at lengths between about 10.5 and 11.5 mm, but not at smaller lengths. Evidence for selective predation with respect to vertebral number is strong in the experiment at the largest prey size (5), but only weak and circumstantial in the experiments at intermediate prey sizes (3 and 4). In experiments at smaller sizes (1 and 2), predation was not selective for either vertebral number or size, and correlations between vertebral number and length were not significant either before or after predation. One possibility, suggested by the absence of size selection in these experiments, is that high predator efficiency overshadowed differences in performance related to vertebral number or size of prey at these small sizes. This possibility is discounted by a very low predation rate in experiment 2. Apparently, fry that will develop 44 or 45 vertebrae perform equally well at these small sizes.

The study of selection in the wild suggested two episodes of selection acting on vertebral number of fry in Holden Lake. The first episode, indicated by the significant difference in vertebral number between the May 21 and 23 samples, occurred when vertebrae were undeveloped in most fry in the lake. This episode followed an influx into the lake of recently hatched fry with a relatively high frequency of low vertebral counts and can be explained by greater mortality among these newly arrived fry than among older fry in the lake. Thus, this episode prob-

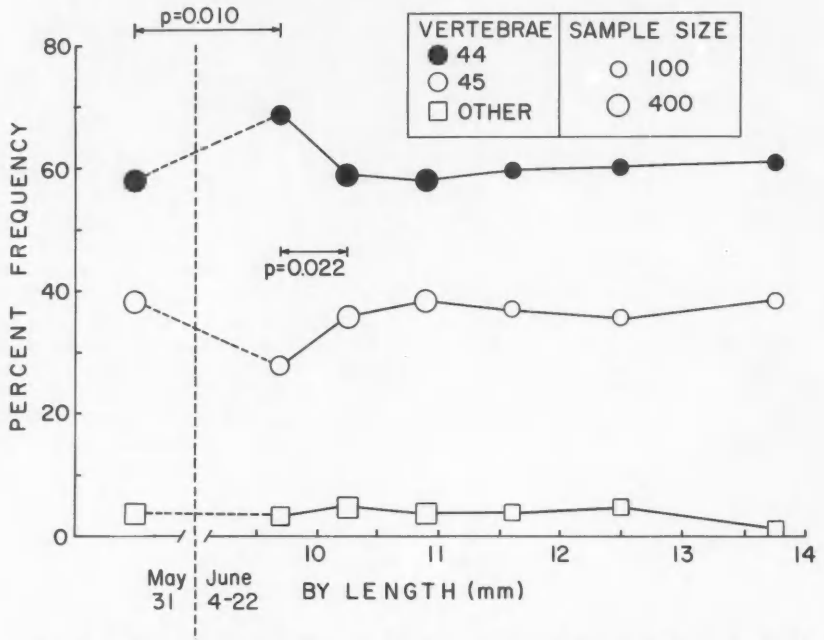

FIG. 6. Vertebral counts of fry in cohort A among fish reared after collection on May 31 (to the left of the broken vertical line) and among those preserved upon collection between June 4 and 22 (to the right of this line). Fry collected in June grouped by length, with fry below 9.3 mm in length omitted due to incomplete vertebral development.

TABLE 10. Vertebral counts of small or large fry in cohort A on May 31 or June 4. Counts are of fry preserved upon collection on June 4, or reared after collection on May 31. Lengths of reared fry are the estimated lengths before rearing.

		Vertebral number (%)					
Date	Size (mm)	<43	44	45	46	*N*	*p*
May 31	8.8–9.3	3.0	55.4	41.1	0.6	168	0.022
June 4	9.3–9.9	2.5	69.3	28.1	0.0	199	
May 31	>9.3	2.0	60.2	36.6	1.2	246	0.56
June 4	>9.9	3.0	54.8	40.7	1.5	135	

ably involved selection related more directly to fry age than to vertebral number or a related feature of the axial segmentation.

On the other hand, the second episode, indicated by changes in vertebral number between May and June samples and among length classes of June samples, occurred just after vertebrae were developed (i.e. countable) in fry and may have involved selection acting directly on vertebral number. In this second episode, selection favoured fry with a low vertebral number (44) at small lengths (9.3–9.9 mm) and those with a high number (45) at slightly greater lengths (9.9–10.6 mm). At even greater lengths (10.6–14.0 mm), survival appeared to be unrelated to vertebral number.

The pattern of selection for vertebral number during this second episode is the same as that observed in *Gasterosteus* (Swain 1986). In *Gasterosteus*, selection also occurred among wild fry only within a narrow "window" of fry lengths, and the optimal vertebral number increased as length increased within this window. Selection in *Gasterosteus* could be attributed to differences among vertebral phenotypes in swimming performance and the ability to avoid predation. *Mylocheilus* fry have not been tested for differences in swimming performance among vertebral phenotypes, but the laboratory experiments described here do demonstrate selective predation for vertebral number of *Mylocheilus* fry.

Both in the laboratory experiments and in the second episode of selection in the wild, selection for vertebral number of *Mylocheilus* fry first occurred at just those lengths when vertebrae were first developed in fry. Possibly, predation was unselective at shorter lengths because fry responses to predators were poorly developed or ineffective at these shorter lengths. However, the predation rate in laboratory experiment 2 was low (Table 1), indicating either a low attack rate by predators or an effective escape response by fry even at these short lengths. Perhaps selection for vertebral number is absent at the shorter lengths, when vertebrae are undeveloped in fry, because the structures directly involved in selection are the vertebrae themselves, rather than some other feature of the axial segmentation such as myomeres or nerves. Possibly, selection is related to an effect of vertebral number on body flexibility, an important factor in the swimming performance of fishes (see Blight 1977).

Selection was consistent between laboratory experiments and the wild in that in both cases a low vertebral number of 44 was favoured at the smallest length when vertebrae were developed in fry. However, this selection appeared to occur at somewhat greater lengths in the laboratory than in the wild. The same discrepancy in length occurred between predation (but not swimming performance) experiments and selection in the wild in *Gasterosteus* (Swain 1986). Because the lengths reported for

predation experiments are those at the end of experiments, and because predation was often size selective, this discrepancy is that expected due to growth during predation experiments and the concentration of mortality at the shorter lengths. Lengths are consistent between the two types of experiment, assuming reasonable growth rates during predation experiments (Swain 1986).

As in the predation experiments with *Gasterosteus* (Swain and Lindsey 1984; Swain 1986), these experiments with *Mylocheilus* also failed to demonstrate an effect of temperature on the selectivity of predation. However, as in the experiments with *Gasterosteus*, this failure might reflect the relatively wide range of lengths among fry exposed to predation in each experiment and a relatively slight shift between temperatures in the lengths at which a particular vertebral number is optimal. Swimming performance experiments with *Gasterosteus* do demonstrate a slight shift between temperatures (15 and 25°C) in the lengths when particular vertebral phenotypes are optimal (Swain 1986).

In *Gasterosteus*, selection was related more directly to the ratio of abdominal to caudal vertebrae than to the total number of vertebrae (Swain 1986). The selection demonstrated here for total vertebral number of *Mylocheilus* fry may also be more directly related to this ratio of abdominal to caudal vertebrae, but these could not practically be distinguished in this study. The use of relative haemal spine length as a criterion, as used in *Gasterosteus*, was not practical in *Mylocheilus*, since differences in spine length near the abdominal/caudal division were not discontinuous in this species as they were in *Gasterosteus*.

Among populations of related fishes, higher mean vertebral number tends to be associated with greater maximum body length (pleomerism) and with higher latitude or colder waters (Jordan's rule). These results with *Mylocheilus*, like those with *Gasterosteus* (Swain 1986), suggest explanations both for Jordan's rule and for pleomerism, in terms of selection acting over a narrow range of fry lengths. Two features of these results are important in this regard. First, in both species, the optimal vertebral number increased as fry length increased. Second, the critical lengths when selection acted on vertebral number were those occurring shortly after hatching, when fry were first free-swimming (*Gasterosteus*) or vertebrae first developed (*Mylocheilus*). Changes in the lengths attained by fry at these early developmental stages will alter the net effect of selection on vertebral number. If fry become free-swimming or first develop vertebrae at greater lengths, they will experience less selection at the small lengths when low vertebral numbers are optimal, and the net selective advantage will be shifted towards higher vertebral numbers in these larger fry. Thus, Jordan's rule and pleomerism might reflect trends in fry length. In fishes, egg and larval or fry sizes do tend to be larger at higher latitudes or in colder waters (e.g. Marshall 1953), providing an explanation for Jordan's rule. A similar correlation between fry and maximum adult lengths has not been tested for, but might be expected, because large females tend to produce large eggs (e.g. Fowler 1972; Gall 1974; Kazakov 1981) and large eggs produce large fry, both at hatching and at complete yolk absorption (e.g. Wallace and Aasjord 1984; Beacham et al. 1985).

Selection appears to act strongly on precise vertebral number at critical fry lengths, at least in *Mylocheilus* and in *Gasterosteus*. Why then does wide variation in vertebral number persist within natural populations of these and other species? One selective mechanism acting to maintain this variation is the change in the direction of selection between different fry lengths. Another may be spatial, seasonal, and annual variation in the fry length or environmental conditions at which selection is most stringent.

The number of vertebrae formed in fish embryos depends partly on water temperatures during egg and embryo development (e.g. Lindsey 1988). This plasticity may be adaptive because the net direction of selection for vertebral number may also depend on water temperatures during these and the early free-swimming stages. For example, hatching size often decreases as developmental temperature increases (e.g. Gray 1929; Alderdice and Forrester 1971; Alderdice and Velsen 1971; Fonds et al. 1974; Peterson et al. 1977; Heming 1982). Thus, a rise in temperature during the spawning season may have caused the decrease during the season in the lengths of fry entering Holden Lake (Fig. 3B). If vertebrae are developed at smaller lengths, fry may face greater exposure to selection at lengths when low vertebral numbers are optimal, shifting the net selective advantage towards lower vertebral numbers later in the season. Hence, the increase in frequency of low vertebral numbers among fry entering the lake late in the season (Table 6) may be adaptive, although the data here are insufficient to determine whether this increase reflects a rise in developmental temperature or a genetic difference between early and late spawners.

The larval or fry stage is a period of great mortality in the life history of fish (e.g. Blaxter 1974). These results in *Mylocheilus* demonstrate that this mortality can be strongly selective and emphasize the potential importance of this life history stage in determining relative fitness.

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Sediment Record of Biogeochemical Responses to Anthropogenic Perturbations of Nutrient Cycles in Lake Ontario[1]

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Two sediment cores collected from the Rochester basin of Lake Ontario were dated with ^{210}Pb and stratigraphic correlation and analyzed to determine whether nutrient accumulation with time was consistent with previous computer-simulated total phosphorus (TP) loadings. Relative increases in TP and nonapatite inorganic phosphorus (NAIP) accumulation were less than the fivefold increase in TP loading from 1800 to 1950 predicted independently from Chapra's simulation model. In addition, increases in TP accumulation occurred mainly after 1940 and the proportion of NAIP relative to TP increased in one core and decreased in the other. Of the nutrients studied, only increases in organic carbon (OC) paralleled the increases in modelled TP loadings. The relative increase in inorganic carbon (IC) was greatest, with accumulation increasing an order of magnitude after 1940 in one core. This large increase in IC, amounting to 20% calcite in recent sediments, was attributed to biologically induced calcite precipitation, a secondary consequence of increased planktonic photosynthetic removal of carbon dioxide that resulted from accelerated eutrophication after 1940 when modelled TP concentrations increased rapidly. Biogenic silica (BSi) accumulation, an indicator of increased diatom production, peaked between 1850 and 1870 when increases in TP and NAIP fluxes were minimal. Results provide evidence that historic biogeochemical responses inferred from OC, IC, and BSi accumulation in the sediment record provide stronger signals of phosphorus enrichment effects than can be inferred directly from changes in accumulation of different forms of phosphorus in the sediment record.

Deux carottes de sédiments prélevées dans le bassin Rochester du lac Ontario ont été datées au ^{210}Pb et par corrélation stratigraphique et analysées afin de déterminer si l'accumulation de matières nutritives en fonction du temps correspondait aux charges de phosphore total (PT) déjà déterminées par simulation informatique. Les augmentations relatives de PT et l'accumulation de phosphore inorganique à l'exclusion de l'apatite (PIEA) étaient inférieures à l'augmentation par un facteur de 5 de la charge de PT prévue de façon indépendante par le modèle de simulation de Chapra pour la période allant de 1800 à 1950. De plus, l'accroissement du taux d'accumulation de PT était surtout noté après 1940 et la proportion de PIEA par rapport au PT augmentait dans l'une des carottes mais diminuait dans l'autre. Des matières nutritives étudiées, seul l'accroissement du carbone organique (CO) présentait un parallèle avec l'accroissement des charges de PT du modèle. L'accroissement relatif du carbone inorganique (CI) était le plus important, son taux d'accumulation augmentant d'un ordre de grandeur après 1940 dans l'une des carottes. Cette importante augmentation du CI, qui représentait jusqu'à 20 % de calcite dans les sédiments récents, a été attribuée à une précipitation de calcite de cause biologique, une conséquence secondaire d'une plus grande disparition du dioxyde de carbone par photosynthèse planctonique qui résultait d'une eutrophisation accélérée des eaux après 1940, moment où les concentrations de PT du modèle augmentaient rapidement. L'accumulation de silice biogène (SiB), qui est un indicateur d'une plus grande production de diatomées, a atteint son maximum entre 1850 et 1870, lorsque les accroissements des flux de PT et de PIEA étaient les moins importants. Les résultats obtenus montrent que les réponses biogéochimiques du passé déduites à partir de l'accumulation de CO, de CI et de SiB dans les sédiments sont de meilleurs indicateurs des effets de l'enrichissement en phosphore que ceux qui peuvent être directement déduits à partir des modifications de l'accumulation des diverses formes de phosphore dans les sédiments.

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Historic total phosphorus (TP) concentrations and loads (from 1800 to 1975) are known only from computer simulations (Chapra 1977). Simulations show bimodal increases in TP amounting to sixfold or sevenfold in Lake Erie, fivefold in Lake Ontario, but only about 75% in Lake Huron. The first inflection in loading is associated with forest clearance and settlement by Europeans in the early 1800s. The second increase began in the 1940s in association with urban sprawl and introduction of phosphate detergents. Management programs instituted in the 1970s to improve sewage treatment and reduce phosphorus inputs reversed a 100-yr trend of increasing TP loads and initiated a period of decreasing TP concentration in Lake Ontario (Kwiatkowski 1982; Dobson 1984).

Historic water quality data are lacking for most of the period of interest; however, the sediment record should reflect historic changes in phosphorus loading. Indeed, based on analysis of one core per lake, Kemp et al. (1972) found postsettlement TP increases in concentration of about 60 or 70% for Lake Huron. However, they found only twofold increases for Lake Erie and Lake Ontario. Analysis of Lake Erie cores (Williams et al. 1976) showed greater postsettlement increases in TP as well as increases in organic phosphorus and nonapatite inorganic phosphorus (NAIP) but increases were still smaller than might be expected from Chapra's simulations.

Only the record of organic carbon (OC) in the sediments appears to correspond closely to the historic TP loads reported by Chapra (1977). Although the temporal pattern of OC increase was similar to that for TP loads, the increase in OC concentration was no more than threefold in Lake Erie or Lake Ontario (Kemp et al. 1972) or about half that expected based on the assumption of a constant stoichiometric relationship between computer-simulated TP loads and OC accumulation in the sediments.

Other sedimentary elements that might vary in response to changing TP load are inorganic carbon (IC) and biogenic silica (BSi). Photosynthetically induced precipitation of calcite which increases with nutrient enrichment (McKenzie 1985) has been reported during summer stratification (Strong and Eadie 1978). An increase in calcium and calcite concentrations in sediments was attributed in part to eutrophication (Mudroch 1983). Previous studies of Lake Erie and Lake Ontario including the Bay of Quinte indicate that increases in BSi concentration occurred early in the sediment record (Schelske et al. 1983, 1985, 1986a), or soon after European settlement. However, the record of BSi accumulation in cores cannot be used to assess historic changes in TP loads. Increased diatom production can deplete soluble silica reserves in the water mass which in turn uncouples diatom production and BSi sedimentation from phosphorus enrichment (Schelske et al. 1983).

We argue here that historic biological effects of phosphorus enrichment in the water column, manifested through increased primary productivity, have affected the biogeochemistry of carbon and silica and hypothesize that the sediment record of IC, OC, and BSi provides insight about historical water chemistry changes not available from other data. Our results show that biogeochemistry of silica and carbon in the Lake Ontario system changed markedly several times since the mid-1800s and that the record of these changes can be resolved in the sediment stratigraphy even though such marked changes would not be predicted from the relatively small changes seen in phosphorus accumulation in the sediment.

Methods

Two box cores and one gravity core (7.5-cm diameter, benthos) were obtained in 1981 from the Rochester basin of Lake Ontario at Stations E30 and G32 at depths of 223 and 158 m, respectively (Fig. 1). Subcores (7.5-cm diameter) obtained from the box corer at both stations were dated with ^{210}Pb. The box core collected at G32 could not be used for early historical comparisons because the sedimentation rate was higher than at E30 and the ^{210}Pb date for the bottom interval of this 40-cm subcore was approximately 1820. Therefore, gravity core G32 (G32-BEN-81) and box core E30 (E30-RNB-81) were the primary cores analyzed for BSi, OC, IC, and different forms of phosphorus.

Cores were extruded and sectioned onboard ship. E30 and G32 box cores were sectioned at 1-cm intervals to 20 cm and at 2-cm intervals to 40 cm. G32-BEN was also sectioned at 1-cm intervals to 20 cm, but at 2-cm intervals to 50 cm and then

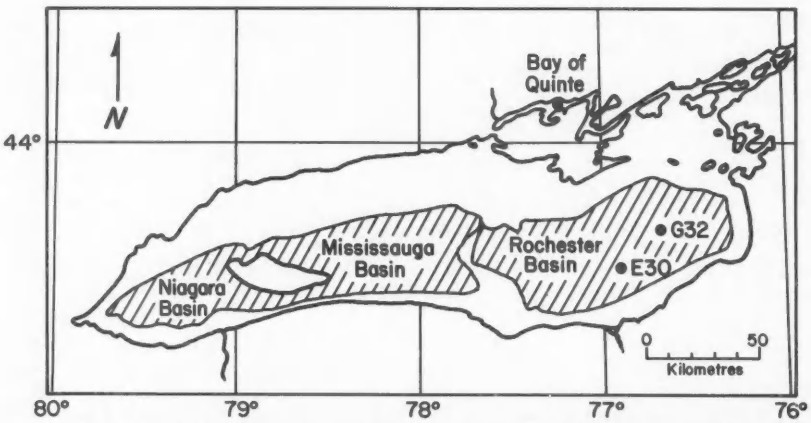

FIG. 1. Depositional basins and station locations in the Rochester basin, Lake Ontario.

at 5-cm intervals to 150 cm. Sections were frozen, weighed, freeze-dried, and weighed again to obtain dry weights and porosities.

Sedimentation rates for E30 and G32 were determined by a combination of ^{210}Pb dating and stratigraphic correlation.

The activity of ^{210}Pb was determined in box cores E30 and G32 by a modified version of a method described by Flynn (1968). Portions of freeze-dried sediment from each interval were spiked with a standard solution of ^{209}Po ($t_{1/2}$ = 103 yr) and extracted for 96 h at 85°C in 10% HCl (v/v) with periodic additions of 30% hydrogen peroxide to oxidize organic matter. For comparison, additional subsamples of material from each section of core G32 were also extracted for 96 h in hot aqua regia. In the aqua regia extraction, the mixture was evaporated to dryness to remove residual nitric acid (which interferes with spontaneous plating) and then reconstituted in 10% HCl (v/v). The activity of the standard ^{209}Po solution (122.2 ± 1.2 dpm·mL^{-1} as of September 17, 1982) was determined by intercomparison with a ^{208}Po reference supplied by the National Bureau of Standards. Each extract was filtered through a 0.45-μm membrane filter and adjusted to a volume of 50 mL with 10% HCl (v/v) to which was added 1.0 g of hydroxylamine hydrochloride to remove the interference of iron with polonium plating. Extracts were adjusted to a final pH of 1.5 ± 0.1 with small additions of concentrated ammonium hydroxide. Polonium isotopes were plated from the extracted solutions for 24 h at 85°C onto polished copper disks backed on one side with Mylar tape.

Activities of ^{209}Po and ^{210}Po were determined by conventional alpha spectroscopy with surface barrier detectors and corrected for radioactive decay of the isotopes, particularly ^{210}Po($t_{1/2}$ = 138.4 d), between the time of plating and counting of the disk. Uncertainties in the activities of ^{210}Po are under 10%. No significant differences were found between the 10% HCl and aqua regia extractions.

The activity of ^{210}Pb should be indistinguishable from that of ^{210}Po because secular equilibrium prevails in most core sections initially and, in addition, several half lives elapsed between collection and extraction of sediment samples.

Average mass sedimentation rates were determined by stratigraphic correlation of the postsettlement BSi peak. The BSi peak was assumed to occur at 1850, or at the date expected for the *Ambrosia* horizon (Kemp and Harper 1976). Because this major peak in BSi concentration was not sharply defined by one sampling interval, the average cumulative dry weight of the two sampling intervals with the largest BSi concentration was used to estimate the average mass sedimentation rate since 1850. The BSi peak was also used to model mass sedimentation rate and nutrient flux in core G32 which was not dated with ^{210}Pb. For this purpose, the peak was set at 1865 which was the date obtained for E30 by ^{210}Pb dating.

Samples for BSi were leached with 1.0% Na_2CO_3 at 85°C and analyzed using previously described procedures (Schelske et al. 1985) including a correction for silicate mineral dissolution (DeMaster 1981).

TP was determined after samples (20–40 mg) were digested in a sulfuric acid/potassium sulfate solution (200 mL of 6 N H_2SO_4, 65 g of K_2SO_4, and 25 mL of 8% HgO diluted to 1000 mL). Two millilitres of this solution, the sediment sample, and 20 mL of double-distilled water were mixed in 50-mL glass digestion tubes, evaporated at 150–200°C for about 1 h, and then digested at 360°C for 22 min. Samples were cooled for approximately 3 min and mixed with 20 mL of distilled water. A portion of each sample was transferred to a 7-mL sample tube, centrifuged, and then analyzed for soluble reactive phosphorus. Extracted phosphorus or NAIP was determined by extracting 0.7–2.0 mg of sample with 10 mL of 0.1 N NaOH in 20-mL plastic vials. The loaded vials were placed on a shaker table and mixed (175 rpm) for approximately 20 h at room temperature, centrifuged, and then analyzed for soluble reactive phosphorus. Soluble reactive phosphorus for both analyses was measured with a Technicon AutoAnalyzer II system (J. M. Malcyzk, Great Lakes Environmental Research Laboratory, NOAA).

OC and IC were determined by dry combustion on a Leco model IR12 carbon determinator. The method uses cupric oxide and an oxygen atmosphere for combustion and infrared detection of the carbon dioxide produced. Total carbon was determined on aliquots of ground dry sediment and OC was determined on separate aliquots that had been treated with concentrated sulfurous acid to remove carbonates by volatilization. IC was determined by difference between results from the two analyses. The system was standardized daily using Lake Ontario sediment of known carbon content. The precision of replicate analyses routinely had a standard error of 3.0%.

All data were converted to units of milligrams per gram of dry sediment or to millimoles per gram of sediment for calculations of atomic ratios. The cumulative inventory of nutrients with core depth was calculated by multiplying the nutrient concentration times the dry sediment weight expressed as grams per square centimetre in each sediment interval and summing the results over the depth of the core.

Results

Mass Sedimentation Rate

Results of models used to interpret ^{210}Pb profiles (Fig. 2) and to obtain age–depth relationships (Fig. 3) and mass sedimentation rates (Fig. 4) are presented in the Discussion along with the assumptions and rationale used to develop the models.

Estimates of average mass sedimentation rate obtained from BSi correlation agreed closely with rates obtained from ^{210}Pb dating and the *Ambrosia* pollen horizon (Table 1). Differences in rates for the two cores from G32 obtained from correlation of the postsettlement BSi peak probably reflect real differences because station location was not fixed accurately for the gravity core (i.e. vessel drifted between sites for box and benthos coring) and point to the need to obtain independent estimates of mass sedimentation rate for every core.

Nutrient Concentration

Both cores showed a major peak in BSi concentration at depth with a less obvious minor peak in more recent sediments (Fig. 5). The major peaks occurred at approximately 1840–1860 in both cores but were deeper in G32 which had a higher sedimentation rate than E30. The maximum concentration was fivefold greater than the minimum concentration in E30 but only about threefold greater than the minimum concentration in G32. Similar major peaks were also present in three cores collected in 1984 in eastern Lake Ontario (Schelske et al. 1986a).

Relative ranges in TP concentration were generally less than those for BSi in both cores (Fig. 5). In both cores, TP concentrations were greater in near-surface sediments than at depth

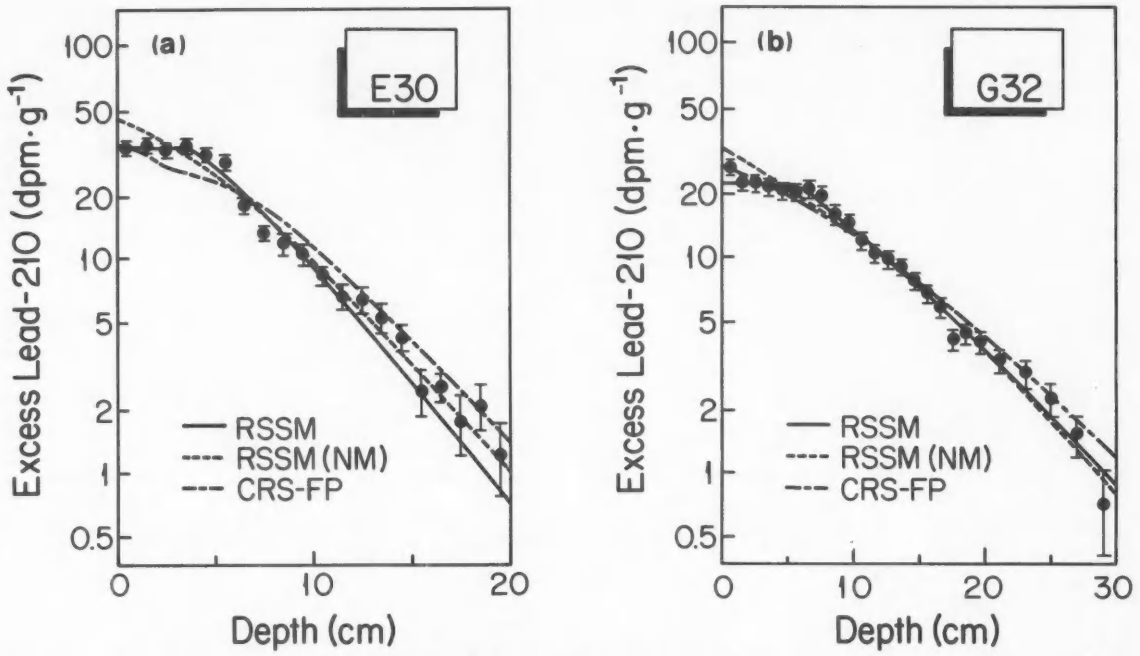

FIG. 2. Excess ^{210}Pb activity with depth for cores (a) E30-RNB-81 and (b) G32-RNB-81. Solid circles with error bars represent excess ^{210}Pb activity. Curves were fitted to excess ^{210}Pb activity using three models: RSSM (rapid steady-state mixing), RSSM-NM (rapid steady-state mixing with effects of mixing removed), and CRS-FP (constant rate of supply – fixed parameter).

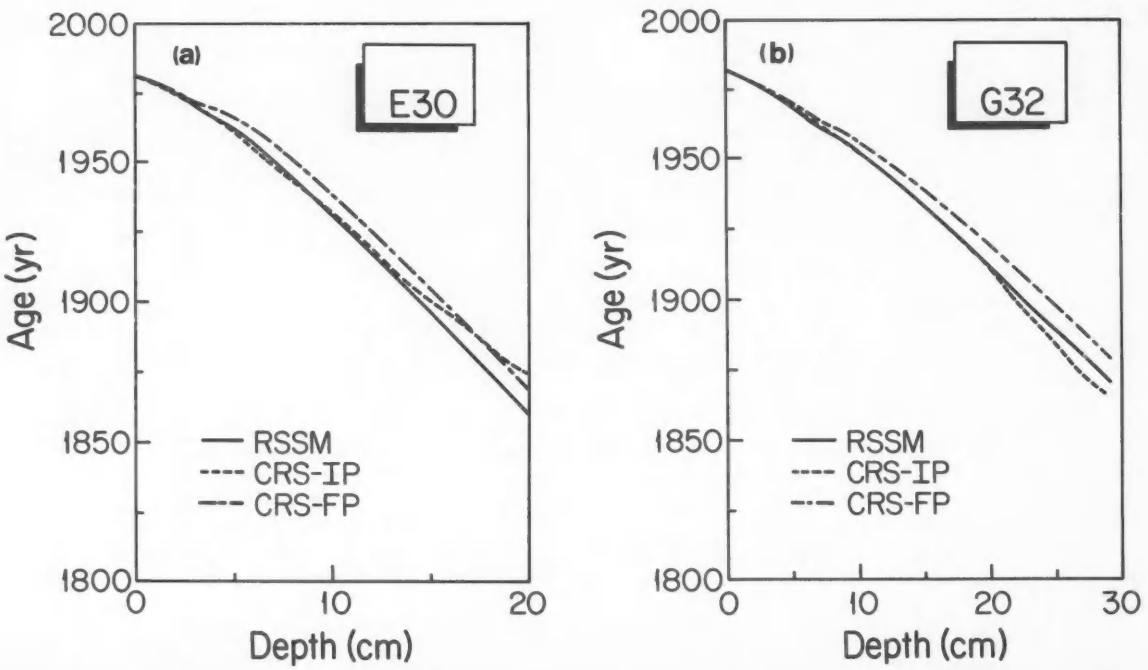

FIG. 3. Depth–time relationships for cores (a) E30-RNB-81 and (b) G32-RNB-81. Curves represent results using RSSM and infinite and fixed parameter CRS models (see text and Fig. 2).

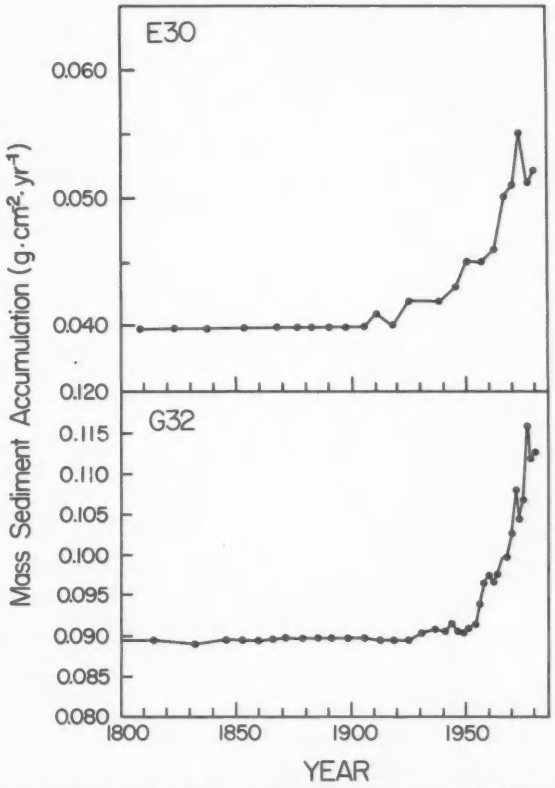

FIG. 4. Mass sedimentation rates in Lake Ontario cores E30-RNB-81 and G32-BEN-81.

TABLE 1. Comparison of mass sedimentation rates ($mg \cdot cm^{-2} \cdot yr^{-1}$) obtained by ^{210}Pb dating, postsettlement BSi peak, and *Ambrosia* pollen horizon. Data in parentheses for the biogenic silica peak are the two estimates used to obtain the average. It was assumed that the biogenic silica peak occurred at 1850, or at the date of the *Ambrosia* horizon obtained from cores collected from 1969-1971 by Kemp and Harper (1976). Mass sedimentation rates for the biogenic silica peaks would be increased by a factor of 1.13 if the date of the peak is assumed to be 1865 as indicated by ^{210}Pb dating.

Station	Core	^{210}Pb	Postsettlement BSi peak	*Ambrosia* horizon
E30	E30-RNB-81	39.1	37.8 (35.6–40.1)	
	22	—	—	35.5
G32	G32-RNB-81	81	71.0 (68.1–73.9)	—
	G32-BEN-81	—	84.7 (82.4–87.0)	—
	27	—	—	81.5

with the exception of the 24–26 cm interval in E30. NAIP profiles for each core were similar to the TP profiles. Similar results were obtained for a core from the Bay of Quinte (Schelske et al. 1985) and for cores from Lake Erie and Lake Michigan (Schelske et al. 1986a).

Of the nutrients studied, the chronology of OC concentration (Fig. 5) was most consistent generally with the expected temporal responses of phytoplankton to patterns of phosphorus enrichment obtained from computer simulations (Chapra 1977). In E30, OC concentrations were relatively constant at the bottom of the core and then showed periods of rapid increase after 1850 and 1940. A similar pattern is evident in G32 but analyses were not run on samples deep enough to obtain data for the relatively low and constant concentrations found below 18 cm in E30. Like OC, concentrations of IC also increased in recent sediments.

Atomic Nutrient Ratios

BSi:TP profiles for both cores show initial major peaks at 18–22 cm for E30 and 40–44 cm for G32 (Fig. 6) which correspond to the major peaks in BSi concentration. In sediments deposited after the major peaks, ratios of BSi:TP and BSi:NAIP decrease. This result is expected if diatom production was silica limited and if rates of diatom dissolution did not change prior to permanent burial to compensate for the reduced flux of diatoms to the sediments.

The largest ratios of NAIP:TP were found in the deepest sediments of one core and in the most recent sediments in the other (Fig. 6). In E30, the only obvious pattern with depth was a tendency for smaller ratios above 19 cm (approximately 1865) with an average ratio of 0.47 below 19 cm or 50% more than the average of 0.31 above 19 cm. In G32, the pattern was reversed. Ratios above 28 cm were >0.36 whereas below that depth, ratios were more variable, with most values being less than the range of values above 28 cm.

Ratios of OC and IC with BSi were greater in near-surface sediments than at depth (Fig. 7). These results may indicate that increased deposition of OC in near-surface sediments resulted from increases in nonsiliceous algal production, provided the bulk of OC was derived from algae.

Nutrient Fluxes

The temporal pattern of TP and BSi flux was similar for cores E30 and G32. BSi accumulation peaked from 1860 to 1870 at 2.5 and 5.5 $mg \cdot cm^{-2} \cdot yr^{-1}$, respectively, for E30 and G32 (Fig. 8). After the peak period, flux in both cores from 1930 to 1940 decreased to half that of the peak period and then increased to a secondary maximum about 1970. Relatively large fluxes for the uppermost interval likely represent input of recently deposited BSi in the zone of active dissolution (Schelske et al. 1983). An increase in TP flux for the two cores (Fig. 9) corresponding with the increased flux of BSi in 1860–1870 was not obvious. An increase in TP flux about 1890 represented by one sample in each core only weakly indicated increased TP fluxes before 1900.

Patterns in NAIP fluxes were different for the two cores. Pre-1850 fluxes at E30 were among the largest fluxes obtained whereas at G32, pre-1850 fluxes were among the smallest (Fig. 10). Like TP fluxes, patterns in NAIP flux were inconsistent with increases expected from modelled TP concentration.

The temporal pattern of increases in OC and IC fluxes was similar for cores E30 and G32. OC flux increased gradually beginning about 1880 until about 1950 when it increased rapidly until about 1970 (Fig. 11). This pattern corresponds closely with that for modelled TP concentration. OC flux after 1970 was relatively constant. Obvious increases in IC flux in both cores were not found until 1920 (Fig. 12). In core E30, IC increased 10-fold by 1950 relative to the early 1800s and 30-fold at the peak in 1970 whereas the historic increase in IC flux at G32 was only about 10-fold. The maximum IC flux at G32

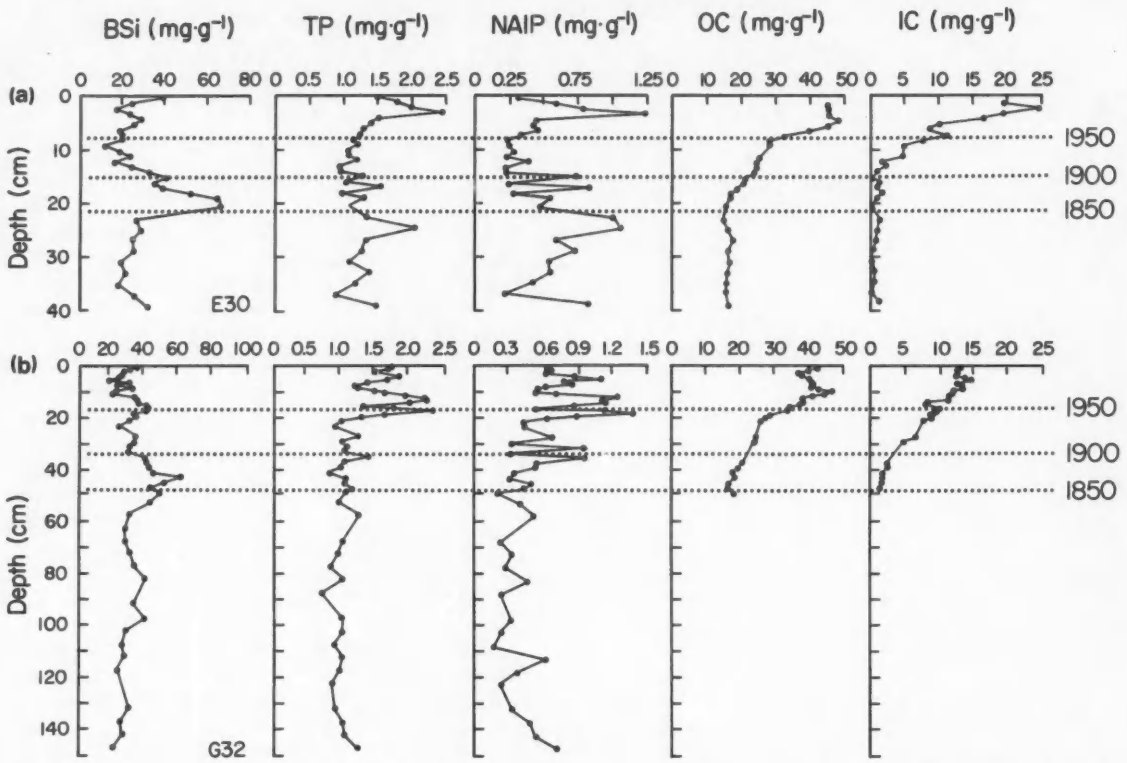

FIG. 5. Concentrations of biogenic silica, total phosphorus, nonapatite inorganic phosphorus, organic carbon, and inorganic carbon in Lake Ontario cores. (a) Box core E30-RNB-81; (b) benthos core G32-BEN-81.

was less than that at E30, a pattern that was not found for any of the other nutrients.

Discussion

^{210}Pb Dating

Profiles of excess ^{210}Pb for E30 and G32 (Fig. 2) were calculated as the difference between total ^{210}Pb and a supported activity which is small and assumed to be constant over the length of the core (E30, 2.45 ± 0.25 dpm·g^{-1}; G32, 2.66 ± 0.25 dpm·g^{-1}). Errors associated with the estimate of unsupported ^{210}Pb are the standard deviations about the mean value over a range of depths at the bottom of the core where the activity of total ^{210}Pb is essentially constant. Error bars in Fig. 2 indicate the uncertainty in the estimate of excess ^{210}Pb which is a combination of analytical and counting errors in the determination of total ^{210}Pb and the uncertainty in estimating unsupported ^{210}Pb. The uncertainty is small in the upper portions of the core but increases significantly toward the bottom as excess ^{210}Pb approaches background levels.

Profiles of excess ^{210}Pb exhibit features encountered elsewhere in the Great Lakes: a zone of nearly constant activity extending down from the sediment–water interface. Below this zone (4 cm for E30; 5 cm for G32), activities decrease exponentially with depth (Fig. 2). Previous studies (cf. Robbins 1982) have shown that the depth of this zone is directly related to the range of penetration of macrobenthos in sediments. Tubificid worms were the dominant species (1450 and 3200·m^{-2} in E30 and G32, respectively). These animals redistribute sediments through a conveyor belt feeding mode which introduces both advective and diffusive mixing processes (cf. Robbins 1986). Another important organism present in these sediments was the amphipod *Pontoporeia hoyii*. It appears to mix sediments in a random (eddy diffusive) way over a shallow range of 1–2 cm (cf. Robbins et al. 1979).

Because organisms were present in sufficient densities to cause significant mixing (Robbins 1986), distributions of ^{210}Pb could be interpreted in terms of the rapid steady-state mixing (RSSM) model developed previously. It is important to point out that all models which have been developed to interpret ^{210}Pb profiles (Robbins and Edgington 1975; Robbins et al. 1977, 1978) assume a constant rate of supply (CRS) of excess ^{210}Pb. In large lakes with long hydraulic residence times, this is the most reasonable assumption. The RSSM model assumes that the sedimentation rate is constant and that ^{210}Pb is delivered at a constant rate into a mixed layer of fixed extent which moves upward as sediments are added (Robbins et al. 1979). To apply the RSSM model to the data, a weighted least squares fit was made to determine optimum values of the mixed depth and sedimentation rate. The fit to the excess ^{210}Pb profile is shown in Fig. 2 as is a fit to the profile using the RSSM sedimentation rate with the effect of mixing removed (RSSM-NM). The resulting age–depth relationship for RSSM is given in Fig. 3.

Alternatively, effects of mixing could be disregarded and the flattening of the ^{210}Pb profile ascribed solely to a changing sedimentation rate (Fig. 2). If the ^{210}Pb flux remains constant,

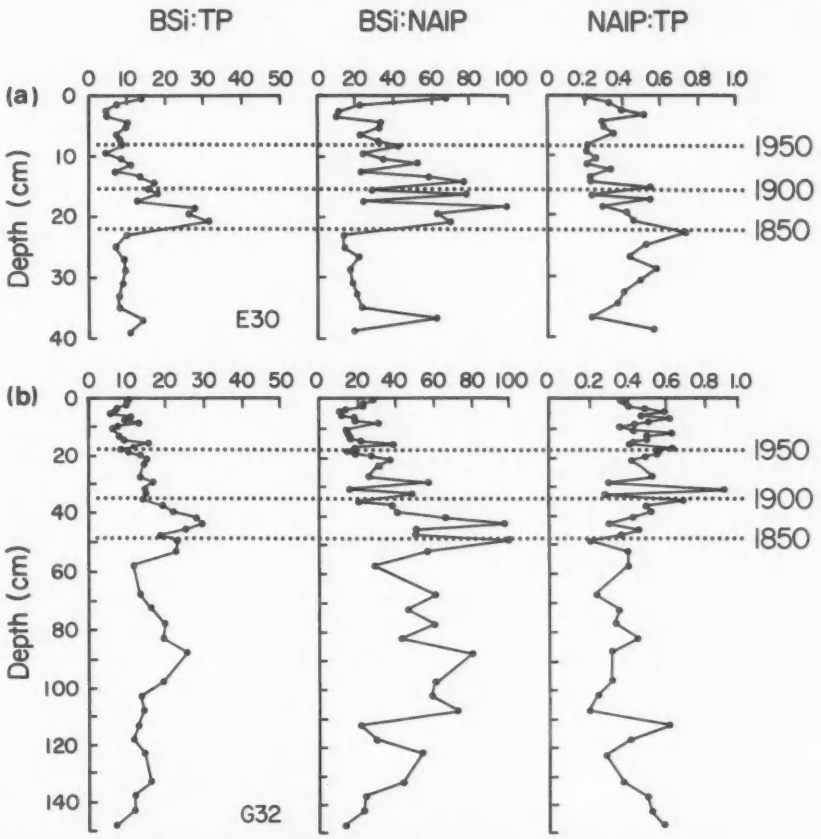

FIG. 6. Atomic ratios of biogenic silica to total phosphorus, biogenic silica to nonapatite inorganic phosphorus, and nonapatite inorganic phosphorus to total phosphorus. (a) Box core E30-RNB-81; (b) benthos core G32-BEN-81.

the relationship between age and depth is given by a straightforward relation described by Goldberg (1963) and later referred to as a constant flux (CF) (Robbins 1978) or CRS model (Appleby and Oldfield 1978). With either of these models, the predicted distribution of excess ^{210}Pb will coincide exactly with the data points because every variation in ^{210}Pb activity is converted into a change in sedimentation rate. Since the CRS model is an infinite parameter (IP) model, processes other than variable sedimentation which contribute to deviations from exponential profiles are conveniently ignored. Moreover, a strictly flat portion on an excess ^{210}Pb profile occurs only for the very special and unlikely circumstance of compensation (see Robbins 1978) where the change of sedimentation rate with sediment depth is exactly matched by radioactive decay. Nevertheless, the result of applying the infinite parameter CRS model (CRS-IP) represents one extreme in the range of assumptions about the nature of the sedimentation process. The age–depth relationship is shown in Fig. 3.

An intermediate treatment, finite parameter CRS model (CRS-FP), attributes some but not all of the flattening to a change in the sedimentation rate (Fig. 2). A correct model would incorporate variable sedimentation and depth-dependent mixing into a single diagenetic equation such as that described by Berner (1980) and modified by Robbins (1986) to include conveyor belt transport processes. For the present purposes, a simpler calculation suffices. Mixing is considered to have a minimal effect on rapidly varying signals such as OC and IC so that profiles of these constituents can be used to reconstruct the time dependence of the net sediment accumulation rate. Other constituents, primarily clay and clay-sized minerals plus a small amount of quartz, are assumed to be supplied at a constant rate, R_s.

To correct for varying concentrations of IC and OC under these conditions the sedimentation rate, $R(g)$, was calculated by

$$(1)\quad R(g) = R_s + R_i(g) + R_o(g)$$

where R_s is the constant base sedimentation rate equal to the RSSM model value, or essentially the sedimentation rate based on the linear portion of the log excess ^{210}Pb curve, and R_i and R_o are the mass sedimentation rates of IC and OC, respectively. All terms are in units of grams per square centimetre per year. The sediment "depth" g is expressed in units of cumulative weight of dry sediment (grams per square centimetre) to remove small compaction effects. The above relationship implicitly assumes that both IC and OC are not subject to diagenesis. If IC and OC have the approximate formulae of $CaCO_3$ and CH_2O, respectively, then

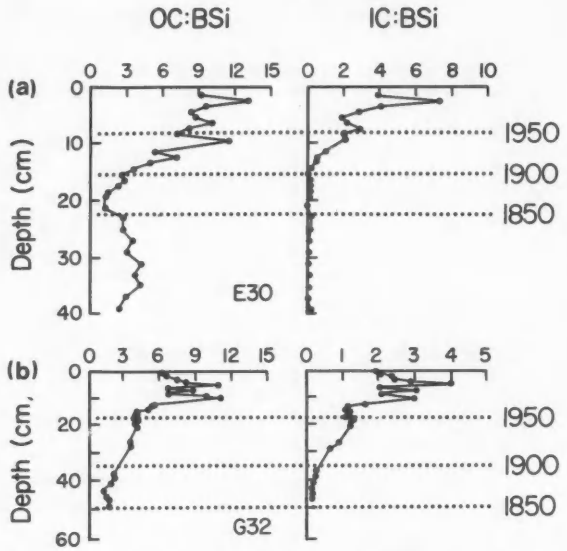

FIG. 7. Atomic ratios of organic carbon to biogenic silica and inorganic carbon to biogenic silica. (a) Box core E30-RNB-81; (b) benthos core G32-BEN-81.

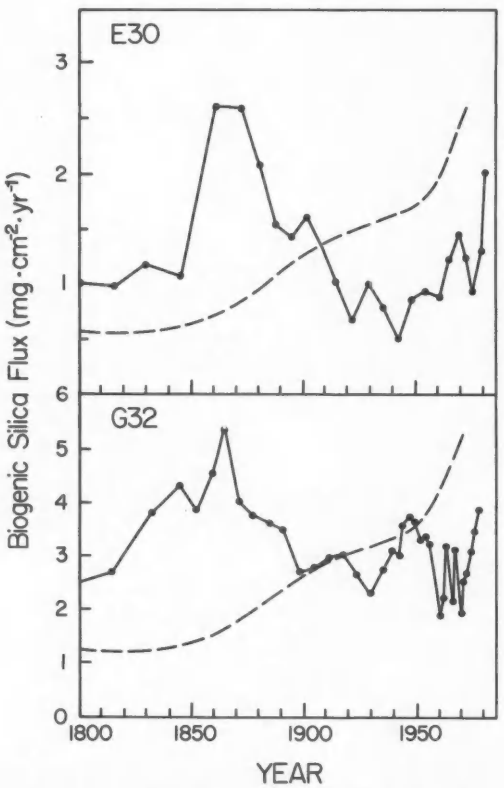

FIG. 8. Flux of biogenic silica in Lake Ontario cores E30-RNB-81 and G32-BEN-81. Broken line is modelled total phosphorus concentration (see Fig. 9).

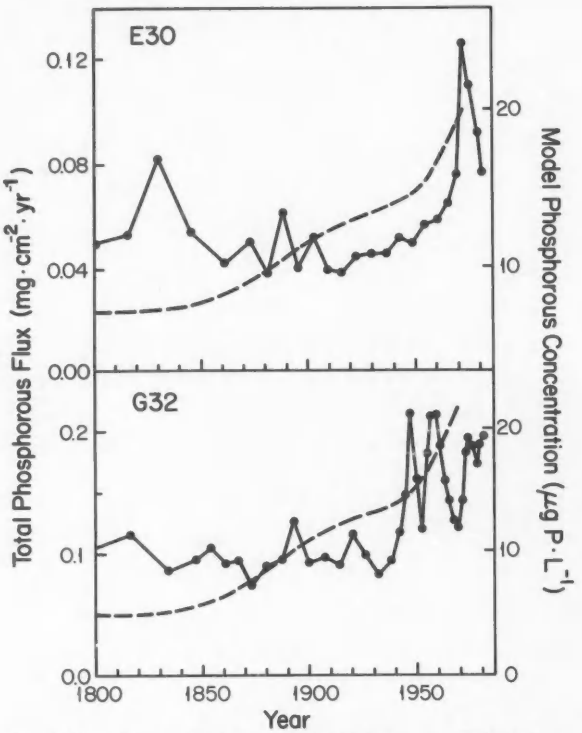

FIG. 9. Flux of total phosphorus in Lake Ontario cores E30-RNB-81 and G32-BEN-81 and computer-simulated total phosphorus concentrations in Lake Ontario from 1850 to 1970 (from Chapra 1977).

$$(2)\quad \begin{aligned} R_i(g) &= 100\,C_iR/12 \\ R_o(g) &= 30C_oR/12 \end{aligned}$$

where C_i and C_o are the measured IC and OC concentrations in grams per gram of dry sediment, respectively. From Equations 1 and 2 it follows that

$$(3)\quad R(g) = R_s/(1\text{-}100C_i/12\text{-}30C_o/12)$$

and the distribution of excess ^{210}Pb is given by

$$(4)\quad A(g) = Fe^{-\lambda t}/R(g)$$

where F is the constant ^{210}Pb flux, t is the time before present, and $\lambda = 0.0311\cdot yr^{-1}$. Since R varies with sediment "depth," the age–depth relationship is given by

$$(5)\quad t = \int_0^g dg/R(g).$$

The value of F was chosen to reproduce the measured standing crop of excess ^{210}Pb in each core. Either the RSSM or the CRS-IP model approximates the data better than the RSSM-NM model in which no mixing is included (Fig. 2). The age–depth relationship (Fig. 3) indicates that the age associated with a given interval of sediment is not strongly model dependent for these cores. The largest differences among estimates occur toward the end of the excess ^{210}Pb profiles where they are within about 7 yr of each other.

The mass flux or net sediment accumulation rate ($R(g)$ vs. t) was based on the CRS-FP model (Fig. 4). For E30 the base sedimentation rate was $0.040\ g\cdot cm^{-2}\cdot yr^{-1}$ and remained essentially constant until 1900 after which it increased to a

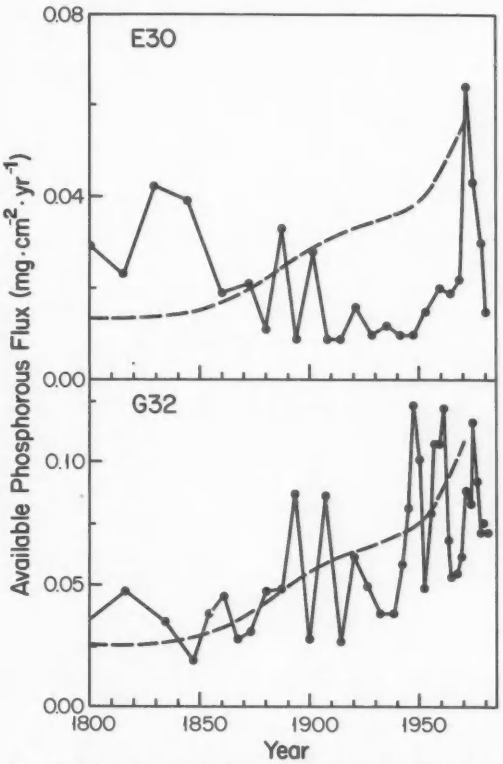

FIG. 10. Flux of nonapatite inorganic phosphorus in Lake Ontario cores E30-RNB-81 and G32-BEN-81. Broken line is modelled total phosphorus concentration (see Fig. 9).

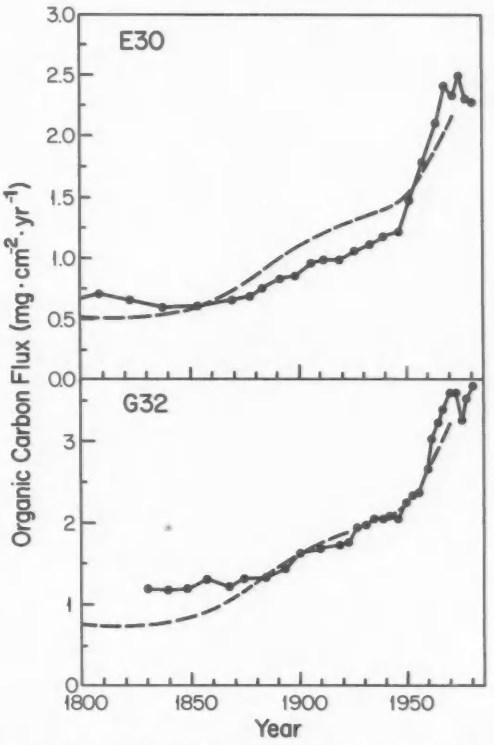

FIG. 11. Flux of organic carbon in Lake Ontario cores E30-RNB-81 and G32-BEN-81. Broken line is modelled total phosphorus concentration (see Fig. 9).

maximum of about 0.055 $g \cdot cm^{-2} \cdot yr^{-1}$. For G32, the base sedimentation rate was about 0.090 $g \cdot cm^{-2} \cdot yr^{-1}$ and also remained constant until about the turn of the century. Thereafter it increased to a maximum of about 0.115 $g \cdot cm^{-2} \cdot yr^{-1}$. Therefore, to the extent that mixing can be ignored, flux of a constituent with concentration C at "depth" g is given by

$$(6) \quad F_c = CR(g)$$

using Equations 1 and 5 above.

Sediment Accumulation and Focusing

Sedimentation rates were obtained using excess ^{210}Pb activity with the CRS-FP model corrected for changes in amount of OC and IC with depth. This correction in ^{210}Pb activity was necessary because carbon-bearing materials constituted a significant portion of the sediment bulk and decreased in concentration over the depth of sediments used for dating (Fig. 5). In lakes with large hydraulic residence times in comparison with ^{210}Pb removal times, the flux of the radionuclide tends to be constant in the presence of changing sedimentation rates (CF model, Robbins 1978; CRS model, Oldfield and Appleby 1984). Thus the effect of increased deposition of carbon-bearing constituents may be to dilute the activity of ^{210}Pb. Alternatively, the observed distributions of OC and IC could be wholly or partly the result of postdepositional processes such as the oxidation of organic materials or dissolution of carbonates. Such diagenetic processes do not affect the dating method if the isotope remains immobilized. The variable sedimentation rates obtained were approximately 17 and 38% greater at G32 and E30, respectively, in the near-surface sediments than at depth (Fig. 4).

Several independent methods are available to make estimates of mass sedimentation rates. The *Ambrosia* horizon along with ^{210}Pb and ^{137}Cs dating can be used independently to date sediments (Robbins et al. 1978), but establishing the *Ambrosia* horizon only provides information for an average postsettlement mass sedimentation rate. Although a direct comparison has not been made, our results (Table 1) suggest that the major BSi peak could be used to define the period of nutrient enrichment resulting from European settlement beginning about 1850. The BSi peak could prove to be a better biostratigraphic marker of the settlement horizon in some cores than the *Ambrosia* horizon because it provides a sharper peak with better time resolution.

As might be expected intuitively, accumulation of nutrients in sediments was related generally to mass sedimentation rate or degree of sediment focusing. This conclusion is supported by the relationship between nutrient accumulation and average mass sedimentation rate. Cumulative inventories of BSi, OC, IC, and TP to the depth of the settlement horizon were 2.24–2.53 times greater in core G32 than in core E30. This is consistent with a 2.23 times greater average mass sedimentation rate in G32 (Table 1). The cumulative inventory of NAIP, however, differed from the other nutrients in that it was 3.33 times greater in G32 than in E30.

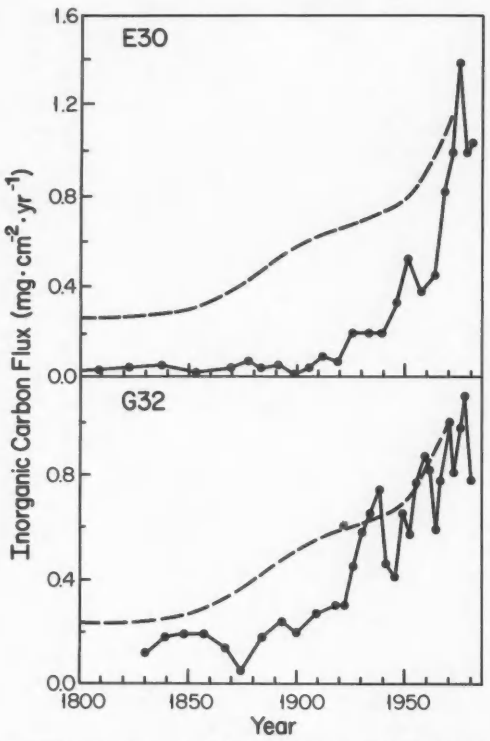

FIG. 12. Flux of inorganic carbon in Lake Ontario cores E30-RNB-81 and G32-BEN-81. Broken line is modelled total phosphorus concentration (see Fig. 9).

Phosphorus and Biogenic Silica Accumulation

The greater relative NAIP accumulation at G32 in combination with the different chronology of NAIP flux at G32 and E30 (Fig. 10) suggests a more complex relationship than simple differences related to sedimentation rate. In G32, NAIP flux and proportion of NAIP increased with time, but these trends were not apparent in the data for E30. The fraction of NAIP relative to TP in the upper 10 cm averaged 46% at G32, about 50% greater than the average of 28% at E30. Williams et al. (1976) and Vernet and Favarger (1982) reported that the proportion of NAIP decreased with sediment depth and attributed this change to an increased proportion of NAIP from anthropogenic sources in recent sediments. In Lake Michigan, large quantities of NAIP are resuspended yearly, primarily during the winter–spring period of thermal mixing (Eadie et al. 1984). In Lake Ontario the NAIP fraction (31–46%) of phosphorus collected in sediment traps (Charlton 1983) was similar to that for NAIP in recent sediments (top 10 cm) at E30 and G32. Williams et al. (1976) concluded that the rate of sedimentation of organic phosphorus and NAIP "was greatest where the most fine-grained sediments were accumulating." One possible explanation, then, for greater NAIP accumulation at G32 is that NAIP is resuspended and deposited with the major part of the fine-grained sediment load in high sedimentation areas in relatively shallow areas of the Rochester basin (Kemp and Harper 1976).

Changes in flux of TP and NAIP for E30 and G32 were not consistent with the fivefold increase in TP inputs from 1800 to 1975 calculated by Chapra (1977). Maximum and minimum yearly fluxes of TP ranged about threefold for both E30 and G32, but twofold increases for both cores occurred between 1940 and 1981 (Fig. 9) when TP loads from sewage inputs increased rapidly (Chapra 1977). Thus, actual loads of phosphorus may have increased less than fivefold or, alternatively, NAIP loads rather than TP loads should have been modelled. Our data for NAIP fluxes do not provide a clear indication of increased flux with time because pre-1850 fluxes were relatively large at E30 and because there was no clear pattern of increasing fluxes between 1850 and 1940 (Fig. 10). Comparison of data for available phosphorus from some Lake Erie cores provided better agreement with computer-simulated TP loads (Williams et al. 1976), but relative increases were still less than those obtained from computer simulations.

By contrast, post-1960 rates of TP sedimentation (Fig. 9) were larger than the average TP sedimentation loss for the lake basin of 0.0250 $mg \cdot cm^{-2} \cdot yr^{-1}$ for the mid-1970s calculated from computer simulation by Chapra and Sonzogni (1979). If 47.7% of the lake bottom is assumed to be in the three major depositional basins (Thomas et al. 1972), then the average rate of TP sedimentation over the depositional area would be 0.0524 $mg \cdot cm^{-2} \cdot yr^{-1}$. This rate is much smaller than measured TP fluxes in recent sediments, especially at G32 (Fig. 9), and intermediate between the recent NAIP fluxes at G32 and E30 (Fig. 10). It is not surprising that the TP loss at G32 with a high sedimentation rate and TP flux was larger than that predicted by the model. In addition, if TP accumulation is related to mass sedimentation rate, greater than average accumulation rates for TP might be expected in the Rochester basin where from 65% (Kemp and Harper 1976) to 80% (Thomas et al. 1972) of the fine-grained sediments are deposited. Data from these two cores therefore indicate better agreement between TP sedimentation losses in the 1970s calculated by Chapra and Sonzogni (1979) and NAIP flux than with TP flux. The twofold or threefold range in rates for the two cores, however, points to the need to analyze cores from additional stations to obtain a better basin-wide average and to analyze cores from other basins to obtain a lake-wide average.

Peaks in BSi accumulation after European settlement have been attributed to depletion of the epilimnetic silica reserve by increased diatom production that resulted from increased phosphorus inputs from the drainage basin of Lake Ontario (Schelske et al. 1983). Increased rates of BSi and siliceous microfossil accumulation (Fig. 8; Stoermer et al. 1985b) indicate that phosphorus limitation for diatom production must have been relaxed after settlement. These results and increased BSi and siliceous microfossil accumulation in the sediments of Lake Superior (Stoermer et al. 1985a) where TP concentrations have not exceeded 5 $\mu g \ P \cdot L^{-1}$ indicate that diatom production can be increased measurably with relatively small phosphorus enrichments. The sensitive response of diatom assemblages to phosphorus enrichment and its relationship to rapid changes in the geochemistry of silica have been addressed previously (Schelske et al. 1986b).

Inorganic Carbon Accumulation

Historic increases in IC accumulation of an order of magnitude or more, however, can be explained only if a combination of biological, physical, and chemical processes changed as the lake became more eutrophic. The most probable cause for the increased flux of IC (Fig. 12) was increased photosyn-

thetic removal of carbon dioxide after 1940 in response to increased TP or NAIP loading (Fig. 9, 10) and an associated increase in pH during summer stratification (Schelske and Callender 1970). In Lake Ontario during the 1970s, epilimnetic calcium concentrations were 40 $mg \cdot L^{-1}$ and summer epilimnetic pH exceeded 8.5 (Dobson 1984). These conditions cause the calcium carbonate saturation product to be exceeded and result in biologically induced precipitation of calcite.

Sediments apparently are nearly saturated with respect to calcium carbonate because near-surface interstitial calcium (0–5 cm) ranged from 50 to 54 $mg \cdot L^{-1}$, larger than concentrations that ranged from 41 to 42 $mg \cdot L^{-1}$ in the overlying water (Weiler 1973). The calcium flux from sediments was estimated to be only 6.25 $mg \cdot m^{-2} \cdot yr^{-1}$ (Weiler 1973), equivalent to only about 0.05% of the calculated calcite flux to the sediments (Fig. 12). These results provide strong evidence that diagenesis of calcium carbonate would not appreciably affect calculated rates of calcite loss to the sediments except possibly in the surface layer of sediments.

In Lake Ontario, calcite precipitation occurs on a lake-wide scale (Strong and Eadie 1978), and calcium and carbonate carbon were highly correlated ($r = 0.985$) in a sediment core (Kemp et al. 1972). Calcium and calcite increased by an order of magnitude in the last 50 yr to more than 20% calcite in cores from the Niagara basin (Mudroch 1983). Also, the downward flux of carbonate during summer stratification is very high (>2.0 $g \cdot m^{-2} \cdot d^{-1}$, Rosa 1985). This summer flux of carbonate (probably calcite) is commensurate with a calcite flux of 56 and 85 $g \cdot m^{-2} \cdot yr^{-1}$, respectively, calculated for recent sediments of E30 and G32.

Other workers have attributed increased calcite concentration in recent sediments of Lake Ontario partly to consequences of eutrophication (Mudroch 1983) or only indirectly to eutrophication through increased carbon dioxide production resulting from decomposition of an increasing load of organic matter which is then preserved as calcite in the sediments (Kemp et al. 1972). Increased dissolution of calcite in the hypolimnetic waters of Lake Erie as the result of eutrophication could have provided a new source of reactants for calcite production in downstream Lake Ontario (Mudroch 1983). If calcite sedimentation of the magnitude we have found is extrapolated over the depositional area of the lake, it is equivalent roughly to the calcium input from the Niagara River (Weiler 1973). The specific mechanism we propose for the 10- to 20-fold increase in IC carbon flux since presettlement is biologically induced precipitation of calcite caused by increased phytoplankton productivity and photosynthetic removal of carbon dioxide that resulted from phosphorus enrichment. Although the proposed mechanism is not unique and was proposed by Minder in 1920 (see Kelts and Hsu 1978; McKenzie 1985), we believe that increased IC flux represents a sediment signal of historic phosphorus enrichment of Lake Ontario waters.

Patterns of Nutrient Accumulation

Four different patterns (signals) of nutrient accumulation in the sediment stratigraphy can thus be used to trace historic responses of phytoplankton communities in Lake Ontario to phosphorus enrichment. The earliest signal, the major BSi peak, occurred between 1850 and 1870. The other three signals occurred much later. An increase in OC flux was evident by 1900 but was most pronounced after 1940. The major increase in IC flux and a secondary peak in BSi accumulation also occurred after 1940. If the phasing of these signals is compared with TP or NAIP flux to the sediments, it is apparent that the first BSi signal reflecting epilimnetic silica depletion was a response to levels of phosphorus enrichment so small that only relatively weak signals of phosphorus flux were found in the sediments. Epilimnetic silica depletion, where mixed layer diatom populations were silica limited in the summer, probably occurred soon after 1850 when open-lake waters contained <10 μg $TP \cdot L^{-1}$. This conclusion is supported by previous results from Lake Michigan (Schelske et al. 1983). Increases in OC flux only resulted from additional phosphorus loading beginning about 1900, and the greatest increase in OC and IC flux occurred after 1940 when simulated TP concentration increased rapidly from a level of about 12–14 $\mu g \cdot L^{-1}$ (Fig. 9). These changes in carbon geochemistry, including an increase of calcite flux to the sediments by at least an order of magnitude, can be attributed to increased phytoplankton productivity. The secondary peak in BSi accumulation could have resulted from the rapid increase in phosphorus loading after 1940 when phosphorus enrichment of lake waters, to levels exceeding 20 μg $TP \cdot L^{-1}$, is thought to have produced water column silica depletion (Schelske et al. 1986b; Stoermer et al. 1985b). After water column silica depletion developed, when silica is <0.4 mg $SiO_2 \cdot L^{-1}$ during the winter–spring convective mixing period, most diatom populations would have been silica limited throughout the year (Tilman et al. 1982). These general trends in nutrient accumulation also provide evidence for dramatic changes in the biogeochemistry of silica and carbon as a result of phytoplankton responses to increased phosphorus loading.

Historic changes in TP concentration in the water column (Chapra 1977) were modelled from assumptions about phosphorus loading from different sources using a term for sediment loss that was linearly proportional to TP concentration in the water. Lack of correspondence between modelled TP concentration and measured TP or NAIP accumulation in sediments can be attributed generally to three factors. First, appropriate parameters could not be modelled because only TP, and not NAIP, data can be obtained from the literature. Because refractory forms of phosphorus are measured as TP, it is not known how much the inputs of biologically available phosphorus increased historically. Second, sediment loss may not be linearly proportional to water column TP concentration (P_w) but may depend on sediment adsorption characteristics (A) which are controlled by $A \cdot P_w^{1/3}$ (Golterman 1980, 1984). Third, historic sediment losses of phosphorus, as discussed below, were probably controlled by a varying combination of physical, chemical, and biological processes.

A major discrepancy between computer-simulated TP concentration and our TP and NAIP fluxes is in the 50-yr period after European settlement beginning about 1850. Simulated TP concentration increased and did not decrease after settlement whereas neither TP nor NAIP accumulation in cores indicates similar trends (Fig. 9, 10). We can only speculate about this discrepancy because we only analyzed two cores and obtained different patterns of NAIP accumulation. Accumulation of phosphorus in sediments then would indicate that P_w concentration was stable or actually decreased after 1850. By contrast, NAIP and TP accumulation increased after 1940 at a faster rate than indicated by modelled TP concentration. Such an increase could be attributed to increased losses associated with increased precipitation of calcite (see Murphy et al. 1983; Koschel et al. 1983), changes in biological turnover rates (Lean et al. 1983) that would be expected to accompany nutrient enrichment, or

both. NAIP and TP fluxes therefore strongly indicate that historic changes in TP concentration cannot be modelled using the assumption that sediment losses are proportional to phosphorus concentration in the water mass.

Both TP and NAIP fluxes were highly variable between some sampling intervals, especially in the period after 1940 (Fig. 9, 10). Variability in TP flux has been noted in a Bay of Quinte core (Warwick 1980; Schelske et al. 1985) and in other Great Lakes cores where the variability results from differences in NAIP concentration among samples (Schelske et al. 1986a). Peaks in phosphorus fluxes after forest clearance might be expected if a major source of loading associated with forest clearance is phosphorus released by the destruction of debris dams in pristine streams (Bilby 1981). Other possible explanations for variability might be postdepositonal mobilization of phosphorus, year-to-year differences in patterns of resuspension or deposition of particulate material during the winter–spring period of thermal mixing, and year-to-year differences in patterns of calcite precipitation. These factors could be influenced by annual fluctuations in weather. In fact, greater than average ice cover and associated reduction in winter–spring thermal mixing in Lake Michigan has been postulated as the mechanism for a short-term decrease in water column TP concentration (Rodgers and Salisbury 1983).

Modelled TP concentration in the water column (Chapra 1977) provides a useful framework on which historic ecosystem changes can be compared. Although assumptions used in obtaining modelled TP concentrations have been questioned, the pattern simulated for Lake Ontario appears to be consistent with the record of organic carbon accumulation in sediments (Fig. 11). Golterman (1984) also found a linear relationship between TP concentration in the water and organic carbon and organic nitrogen accumulation in sediments of Lake Constance. Thus, one of the expected consequences of eutrophication, an increase in production and sedimentation of organic matter, is consistent with the pattern of simulated TP concentration.

In summary, a record of changing biogeochemistry in Lake Ontario during the last 130 yr can be obtained from the stratigraphic analysis of several nutrients in sediment cores. BSi accumulation peaked between 1850 and 1870 in response to hypothesized small increases in phosphorus inputs resulting during early European settlement. The most obvious record of postsettlement change was increased deposition of OC and also increased deposition of IC in the form of calcite, a secondary effect of increased nutrient loading and phytoplankton production. The sediment record for OC and IC corresponds temporally with increased deposition of TP because major changes occurred since 1940 when the major increases in TP deposition were found. Relative changes in TP or NAIP accumulation, however, were less than those for OC, IC, or BSi and, unlike the other nutrients, differed between cores, suggesting that system responses to increased P loading cannot be predicted entirely on stoichiometric relationships between phosphorus and other nutrients. The relationship is complicated because rate constants of phosphorus recycling by organisms (Lean et al. 1983), co-precipitation of phosphorus with calcite (Murphy et al. 1983), or sorption of phosphorus to particles that remain suspended in the water column (Eadie et al. 1984) may have been modified during the changing trophic history of Lake Ontario.

Even though our results confirm that the trophic history can be inferred from the sediment record, discrepancies in ratios of NAIP:TP and differences in NAIP storage and recent IC flux between cores point to a lack of complete understanding about the factors that interact to produce the sedimentary record. Additional cores should be analyzed to determine how patterns of nutrient sedimentation and accumulation vary over depositional environments and to test hypotheses suggested by our results.

Acknowledgments

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NOTES

Ecological Significance of Differences in Frequency of White Fin Margins Among Four Brown Trout (*Salmo trutta*) Populations

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Taugbøl, T., J. Skurdal, and R. Andersen. 1988. Ecological significance of differences in frequency of white fin margins among four brown trout (*Salmo trutta*) populations. Can. J. Fish. Aquat. Sci. 45: 1304–1309.

The frequency of distinct white fin margins was investigated in four brown trout (*Salmo trutta*) populations, living in the small stream Austadbekken (anadromous and stream resident) and in Lake Selura (lake and stream spawners). The frequency varied within and among populations. White margins were most common among parr and mature residents from Austadbekken. Sea trout had very low frequencies compared with parr and mature residents from the same population. Among stream spawners from Lake Selura (which stay the first 0–2 yr in the stream), white margins were less frequent than in Austadbekken; lake spawners (which spend their entire life in the lake) had even fewer. While a previous author stated that white fin margins were under genetic control, our observations indicate that the environment can affect the coloration. We hypothesize that white fin margins play a role in territorial behavior and are developed strongly where territorial behavior is pronounced, i.e. in stream- and river-dwelling trout. There may be a selective pressure, favoring easily seen signals showing that a territory is occupied so that energy-demanding fights can be avoided. In open sea and estuaries where predators are frequent, conspicuous fin margins may be a disadvantage due to greater predation risks.

On a étudié la fréquence de marges de nageoire blanches chez quatre populations de truite brune (*Salmo trutta*) peuplant le petit ruisseau Austadbekken (populations anadrome et sédentaire) et le lac Selura (géniteurs lacustres et fluvaux). On a observé une variation interpopulation et intrapopulation. La présence de marges blanches était plus commune chez les tacons et les truites matures sédentaires de l'Austadbekken. Les individus marins montraient une très faible fréquence par rapport aux tacons et aux truites matures d'une même population. Chez les géniteurs fluviaux du lac Selura (qui restent jusqu'à 2 ans après leur naissance dans le cours d'eau), la présence de marges blanches était moins élevée que dans l'Austadbekken tandis que les Géniteurs lacustres (qui n'émigrent pas) montraient une incidence encore plus faible. Selon des données publiées, la présence de marges de nageoire blanches est sous contrôle génétique; par contre, les données obtenues au cours de la présente étude révèlent que l'environnement peut influer sur la coloration. Les auteurs formulent l'hypothèse que la marge de nageoire blanche joue un rôle dans le comportenent territorial; elle est plus évidente chez les individus é comportement territorial développé, soit les truites de ruisseau et de rivière. Il peut exister une pression de sélection qui favoriserait l'existence de signaux facilement visibles montrant qu'un territoire est occupé de façon à éviter des combats exigeant une dépense d'énergie. En haute mer et dans les estuaires où des prédateurs sont souvent présents, la présence d'une marge de nageoire évidente peut représenter un désavantage à cause des risques plus élevés de prédation.

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Brown trout (*Salmo trutta*) live in diverse habitats, including streams, rivers, lakes, and open sea. There are various forms of brown trout, each adapted to its own environment (Jonsson 1981). The various forms may differ in body form, color, and physiological characteristics, and many of the differences observed are genetically determined (Jonsson 1982). One conspicuous difference between stream- or river-dwelling brown trout and lake-dwelling brown trout is the presence of white fin margins: frequent in the former, but occuring only in minor extent in the latter (Sømme 1948; Alm 1949). Alm (1949)

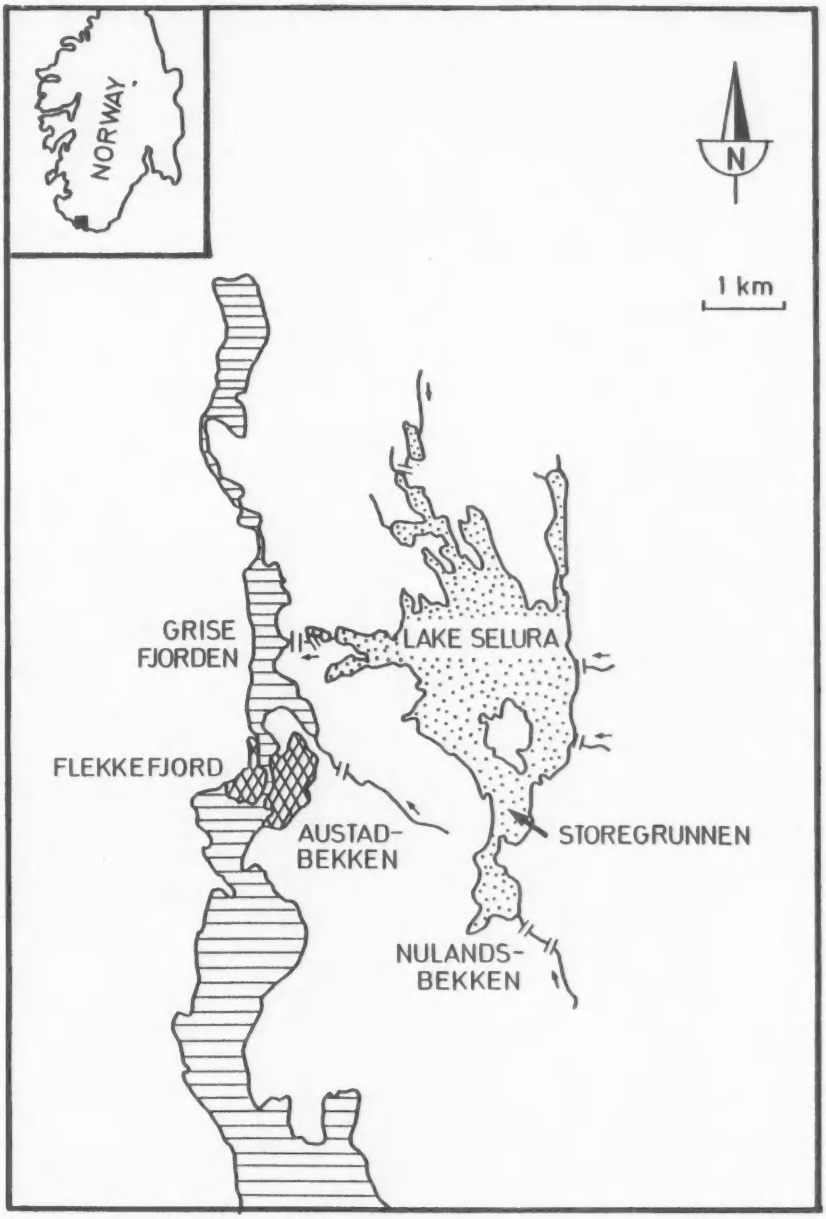

FIG. 1. Study area.

showed by breeding experiments that the presence of white fin margins was chiefly due to genetic factors. Progeny of river- and lake-dwelling brown trout, when reared under similar conditions, exhibited the same differences in frequency of white fin margins as the parental fish.

We investigated the frequency of white fin margins in four brown trout populations, two living in the small stream Austadbekken (above and below an impassable waterfall in an anadromous system) and two living in Lake Selura (lake and stream spawners). The aim of this study was to investigate how the frequency of white fin margins varied among and within populations, and further, to discuss the ecological significance of this characteristic.

Methods

Study area and fish stocks — Austadbekken and Lake Selura are situated near Flekkefjord in the county of Vest-Agder, southwestern Norway (Fig. 1). The stream Austadbekken is 1.7 km long and drains into Grisefjorden which is part of a fjordsystem connected to the North Sea. Austadbekken is a floodstream with great variations in waterflow. At normal flow

TABLE 1. Sample sizes and length ranges (cm) of parr, residents, and sea trout caught in the four locations during October.

Location	Parr		Residents		Sea trout	
	N	Length	*N*	Length	*N*	Length
Austadbekken, upper	93	7–16	105	9–30		
Austadbekken, lower	410	7–17	273	7–30	59	17–59
Nulandsbekken	38	6–21	542	9–35		
Storegrunnen	85	12–24	259	12–38		

the width is 0.9–3.0 m and the depth 0.2–1.1 m. There are two fish species in the stream: brown trout and eel (*Anguilla anguilla*). The number of eels is small. Approximately 600 m above the stream mouth there is an impassable waterfall which functions as an upstream migration barrier between two brown trout populations. Below the waterfall there are parr, mature residents, and sea trout all belonging to the same population (Taugbøl 1985). The parr stage is spent in the stream. At age 2+ or 3+, some parr smoltify and go to the sea as sea trout. The sea trout occupy the stream only during the spawning period and return to the sea immediately after spawning. Parr that do not smoltify mature sexually as residents and stay in the stream for their entire life span. Below the waterfall the stream consists of ripples and small waterfalls together with pools, back-eddies, and slow-floating regions. The bottom consists of gravel, stones, and mud. The stream is lined with riparian vegetation.

Above the waterfall the population consists of parr and resident brown trout only. Half the investigated section resembles the stream below the waterfall. The other half is canalized with

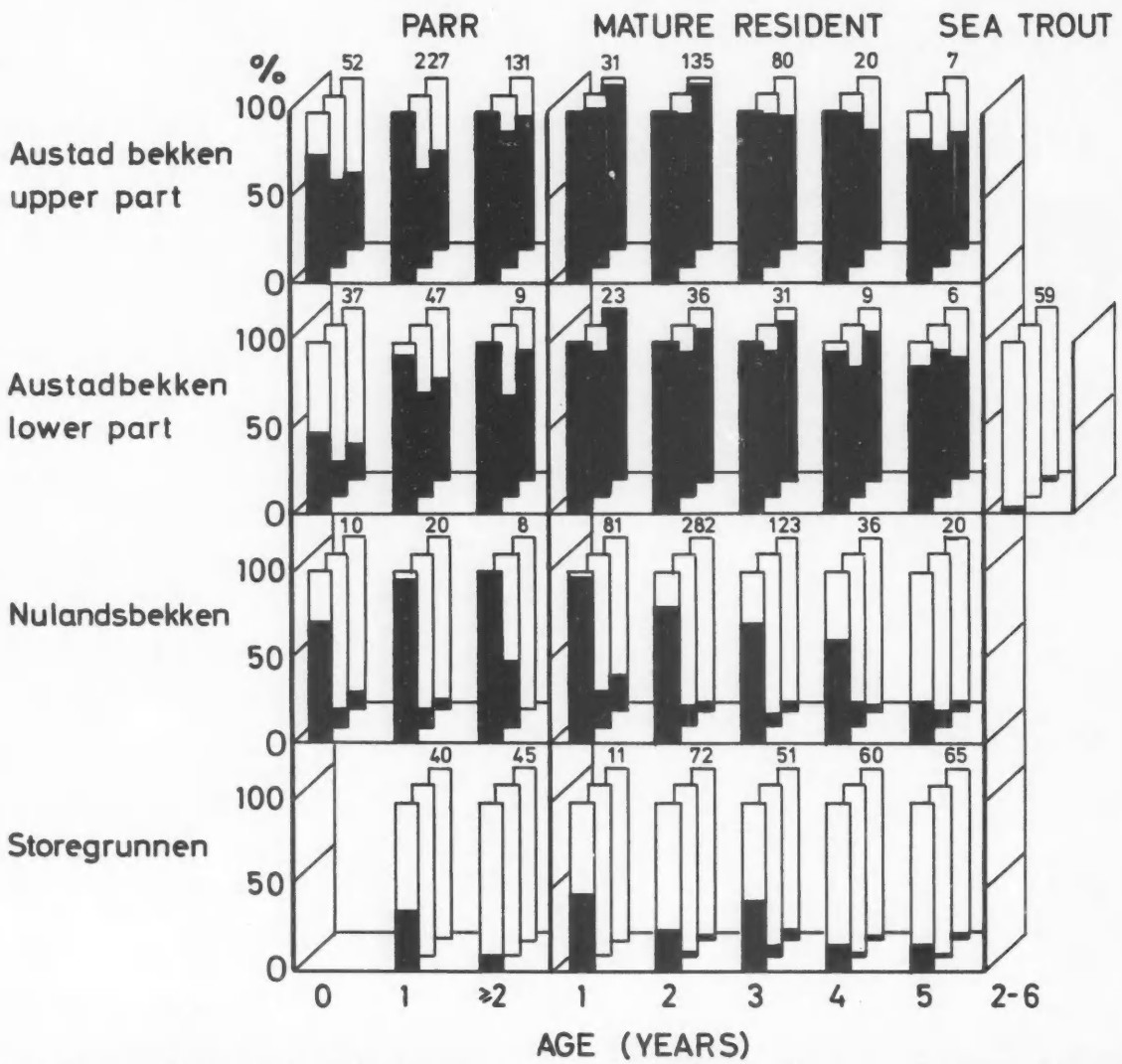

FIG. 2. Frequency of distinct white fin margins in three types of brown trout, by age, from four different locations. Front bar: anal fin; middle bar: pelvic fins; back bar: dorsal fin. Black square is proportion of white fin margins. Numbers above the bars are sample size.

TABLE 2. Tests of homogeneity (χ^2 test) of white fin margin frequencies among parr in age groups 0–2 (df = 2) and mature residents in age groups 1–5 (df = 4), by location. NS = not significant, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

		Anal		Pelvic		Dorsal	
Location		χ^2	p	χ^2	p	χ^2	p
Austadbekken, upper	Parr	15.08	***	2.07	NS	5.98	NS
	Mature			2.09	NS	10.28	*
Austadbekken, lower	Parr	106.95	***	36.60	***	45.92	***
	Mature			0.97	NS	2.78	NS
Nulandsbekken	Parr	5.62	NS	3.59	NS	0.90	NS
	Mature	53.71	***	7.09	NS	24.83	***
Storegrunnen	Parr	8.65	*				
	Mature	14.02	**	2.85	NS	2.11	NS

TABLE 3. Tests of homogeneity (χ^2 test) of white fin margin frequencies between parr and mature brown trout of two age-classes, by location. NS = not significant, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ (df = 1).

		Anal		Pelvic		Dorsal	
Location	Age	χ^2	p	χ^2	p	χ^2	p
Austadbekken, upper	1			8.21	**	7.41	**
	2			0.77	NS	2.47	NS
Austadbekken, lower	1	2.80	NS	6.70	**	18.07	***
	2	0.00	NS	8.88	**	7.66	**
Nulandsbekken	1	0.07	NS	1.04	NS	2.20	NS
	2	2.24	NS	4.09	*	0.29	NS
Storegrunnen	1	0.40	NS				
	2	4.72	*	1.27	NS	1.27	NS

stone-walls and gravel bottom. The habitat here is much more homogeneous but the stone walls still support numerous hiding places for the trout (Taugbøl 1985).

Lake Selura (surface area 510 ha, maximum depth 65 m, elevation 32 m above sea level) also drains into Grisefjorden (Fig. 1). In addition to brown trout the lake harbours Arctic char (*Salvelinus alpinus*) and eel. The brown trout and the Arctic char are lake resident; migration from the sea by the salmonids is prevented by a waterfall in the outlet river. One brown trout population spawns in the inlet stream Nulandsbekken (Fig. 1). The spawning and nursery areas are restricted to the lower 160 m section of this stream. The width of the stream is up to 6 m, and the depth varies from 0.2 to 2.0 m. The stream bottom consists mainly of gravel and stones and the stream is lined with riparian vegetation. The lower 60 m is the deeper, slow-floating part. The parr stay in the stream 1–2 yr before migrating into the lake. Mature fish occupy the stream only during the spawning period. Another trout population spawns in the littoral zone of the lake at Storegrunnen (Fig. 1). This spawning area is approximately 50 m long, 1–4 m wide, and 4–5 m deep. The brown trout from this population spend their whole life in the lake. Marking experiments have shown that straying between the two populations is insignificant (Andersen 1980).

Sampling and data analysis — We caught a total of 1805 brown trout (Table 1) by electrofishing in the streams and by gill-netting in the lake. We fished during October (the spawning period) 1982 and 1983 in Austadbekken and October 1978 in Nulandsbekken and at Storegrunnen. At Storegrunnen we were unable to catch parr in age group 0.

We recorded the color of the anal, pelvic, pectoral, and dorsal fins and distinguished between (1) white fin margins (i.e. distinct white fin margins, often with a black edge outermost) and (2) indistinct white margins or lack of white margins. This is a subjective classification and it could sometimes be difficult to distinguish between distinct and indistinct. In category (2), indistinct or lack of white margins, the frequency of "indistinct" varied from 0 to 80%, but typically it was less than 50%. No fish had white margins on the pectoral fins, so these fins were omitted from the analysis. There were no differences between sexes, so these are treated together. No differences could be detected between different sections within lower Austadbekken, upper Austadbekken, and Nulandsbekken, respectively. Frequency differences were tested by chi-square analysis.

Results

Among parr the frequency of white fin margins was higher in the older fish at all locations except Storegrunnen (Fig. 2). This was, however, not significant for all fins (Table 2). Among parr from Storegrunnen the frequency of white margins on the anal fin was significantly higher in the younger parr (Table 2).

Among mature residents from Austadbekken the frequency of white fin margins was high and almost constant in all age groups (Fig. 2). There seemed to be a lower frequency of white fin margins in the older age groups, which was significant, however, only for dorsal fins of the landlocked fish of upper Austadbekken (Table 2). Among mature brown trout from Lake Selura (both lake and stream spawners) the frequency of white anal fin margins declined gradually and significantly with increasing age (Fig. 2; Table 2).

When significant frequency differences occurred between parr and mature brown trout of the same age, the mature fish generally had the greater proportion of white fin margins (except for the anal and pelvic fins in age group 2 from Nulandsbekken) (Fig. 2; Table 3).

There were marked differences among parr from the different locations (Fig. 2; Table 4). Compared with parr from the lower part of Austadbekken the parr in the upper part had significantly higher frequencies of white fin margins on all fins in age group 0 and on the dorsal fin in age group 1. Furthermore, the parr from the lower part of Austadbekken had a significantly higher frequency of white fin margins on the pelvic fins in age group 1 and on the dorsal fin in age groups 1 and 2 compared with parr from Nulandsbekken. These, in turn, had much higher frequencies of white margins on anal and pelvic fins than the parr from Storegrunnen.

There were no significant differences between mature residents from the upper and lower part of Austadbekken (Fig. 2; Table 4). Mature residents from the lower part of Austadbekken had significantly higher frequencies of white fin margins in all fins and age groups, except for the anal fin of age group 1, compared with mature brown trout from Nulandsbekken. Furthermore, there was a significantly higher frequency of white fin margins on the anal fin (age groups 1–4) and the pelvic fins (age groups 2 and 4) of mature stream spawners compared with lake spawners in Lake Selura.

Although distinct white margins dominated among parr over age 0 and mature residents from the lower part of Austadbekken, sea trout from the same population exhibited very low

TABLE 4. Tests of homogeneity (χ^2 test) of white fin margin frequencies in parr and mature brown trout between different locations, by age-class. NS = not significant, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ (df = 1).

			Anal		Pelvic		Dorsal	
Location		Age	χ^2	p	χ^2	p	χ^2	p
Austadbekken, upper	Parr	0	7.76	**	10.15	**	6.03	*
vs.		1	4.23	*	0.10	NS	1.21	NS
Austadbekken, lower		2	0.07	NS	0.43	NS	0.06	NS
	Mature	1			0.65	NS	0.05	NS
		2	0.27	NS	0.75	NS	1.43	NS
		3			0.19	NS	0.35	NS
		4	0.47	NS	0.73	NS	1.27	NS
		5	0.01	NS	0.66	NS	0.03	NS
Austadbekken, lower	Parr	0	1.91	NS	0.49	NS	0.49	NS
vs.		1	0.28	NS	18.57	***	20.16	***
Nulandsbekken		2	0.06	NS	2.94	NS	19.60	***
	Mature	1	1.18	NS	39.33	NS	57.12	***
		2	32.13	***	196.49	***	301.81	***
		3	28.46	***	120.90	***	148.60	***
		4	7.53	**	20.91	***	39.85	***
		5	7.92	**	14.26	***	6.80	**
Nulandsbekken	Parr	1	19.39	***	4.14	*	2.03	NS
vs.		2	32.19	***	17.89	***		
Storegrunnen								
	Mature	1	28.37	***	2.63	NS	2.43	NS
		2	73.17	***	5.97	*	0.10	NS
		3	13.38	***	0.12	NS	0.00	NS
		4	20.00	***	5.74	*	0.14	NS
		5	0.65	NS	3.22	NS	1.08	NS

frequencies (Fig. 2). The age 0 parr were intermediate between the sea trout and the age 0 parr from the upper Austadbekken.

Discussion

Among stream-dwelling parr from upper and lower Austadbekken the frequency of white fin margins was significantly higher in the older age groups, and mature stream dwellers had a higher frequency than parr of the same age. Thus, the development of the white fin margins appears associated with sexual maturity. This finding supports the evidence reported by Alm (1949).

Most parr and all residents from Austadbekken had much higher frequencies of white fin margins than sea trout belonging to the same population. For example, all 1+ and 2+ parr (includes those which become 2- and 3-yr smolts the following spring) had white margins on the anal fin. Almost none of the sea trout had them, and hence a loss of white margins must occur. Among brown trout from Nulandsbekken, which migrate into Lake Selura at the age of 1–2 yr, only the anal fin had a high frequency of white margins and the frequency declined sharply with age. Among brown trout from Storegrunnen, which stay in Lake Selura throughout life, the frequency of white margins was very low. These observations clearly indicate a connection between the habitat occupied by the trout and the coloration of the fin margins. The frequency of white fin margins differed greatly both among and within populations. Alm (1949) showed by breeding experiments that white fin margin differences between stream- and lake-dwelling brown trout mainly were under genetic control. The ability to express white fin margins may be genetically controlled, but environmental factors clearly have an effect on the coloration, as we observe a loss of white margins by age among mature trout from Nulandsbekken and also a loss from parr to sea trout in lower Austadbekken. Selective mortality for trout having white margins (e.g. greater predation risk) could also explain a decline of white margins by age. However, this is not likely in Lake Selura where no predation on brown trout by other fish species occurs. Cannibalism has not been observed (Vøllestad and Andersen 1985). Predation by fish on migrating salmonid smolts in estuaries and before reaching marine water is well documented (Piggins 1959; Larsson 1985; Hvidsten and Møkkelgjerd 1987). Conspicuous white margins on fins of sea trout may therefore be a disadvantage because of increased vulnerability to predators.

Why are white fin margins strongly developed in some brown trout populations, i.e. what is the ecological significance of this characteristic? Arctic char, especially during the spawning period, have distinct white fin margins. According to Fabricius (1953), these may be territorial signals releasing escape or submissive behavior in conspecifics. We hypothesize that white fin margins play a similar role in territorial behavior of brown trout. Young trout are territorial (Lindroth 1955; Kalleberg 1958) and this behavior is also present among older fish (Miller 1957; LeCren 1965). The reason why white margins are typical only in stream- and river-dwelling trout populations may be associated with the fact that fish in these locations live very close to each other. From the viewpoint of evolutionary biology, reduction of interspecific competition would minimize wastage of time and energy. This would induce a selective pressure for easily seen signals showing that a territory is occupied, so that energy-demanding fights can be avoided. By having the signals on the fins the fish are able to both show or hide signals when needed, just by changing the position of the fins.

The reason why white margins are more developed in Austadbekken compared with Nulandsbekken may be associated with the fact that in Austadbekken, all size groups and year-classes stay in the stream the whole year, increasing competition and the need of territorial signals. In Nulandsbekken, only parr of age 0–2 stay in the stream the whole year. Mature fish occupy the stream only during the spawning period.

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Environmental Influence on Activities and Foreign-Particle Binding by Hemocytes of American Oysters, *Crassostrea virginica*

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American oysters (*Crassostrea virginia*) from an estuarine and an oceanic habitat were held in the laboratory under various temperature and salinity regimes. After a month, their hemocytes were withdrawn from the adductor muscle and measured in vitro for time to spreading (TTS), time to spreading after an acute salinity increase (TTS + 12), rate of locomotion (ROL), and binding of fluorescent microspheres (beads). Bead binding was compared with binding of bacteria (*Vibrio parahemolyticus*). TTS and TTS + 12 measurements were negatively correlated with temperature whereas ROL and bead binding measurements were positively correlated with temperature. An acute rise in salinity (TTS + 12) increased the time required for hemocyte spreading. Spreading was faster, however, at higher salinities for acclimated oysters from both habitats. This finding contradicted previous results for estuarine oyster hemocytes and emphasized the role of environmental history and/or seasonality on hemocyte responses. Oceanic oysters from a less variable environment showed hemocytic responses consistent with previous studies.

Des huîtres américaines (*Crassostrea virginica*) provenant d'habitats estuarien et océanique ont été conservées en laboratoire dans différentes conditions de température et de salinité. Après un mois, on a prélevé des globules sanguins du muscle adducteur et on a mesuré *in vitro* le temps d'étalement (TTS), le temps d'étalement après une augmentation importante de la salinité (TTS + 12), la vitesse de déplacement et la fixation de microsphères fluorescentes (billes). La fixation à des billes a été comparée à la fixation de bactéries (*Vibrio parahemolyticus*). On a relevé une corrélation négative entre le TTS et le TTS + 12 et la température tandis que la vitesse de déplacement et la fixation à des billes présentaient une corrélation positive avec la température. Une augmentation importante de la salinité (TTS + 12) a augmenté le temps nécessaire à l'étalement des globules. L'étalement était plus rapide, cependant, à salinité élevée dans le cas des huîtres acclimatées fréquentant les deux types d'habitat. Cette observation contredit les résultats antérieurs dans le cas des globules d'huître estuarienne et met l'accent sur le rôle des antécédents environnementaux et (ou) de la saison sur la réaction des globules. La réaction

des globules sanguins d'huîtres océaniques, provenant d'un milieu moins variable, était conforme aux résultats d'études antérieures.

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Disease is a significant factor in the oyster fisheries of the North American Atlantic seaboard and several programs are under way to develop and utilize disease-resistant stocks for private and public cultivation efforts (Haskin and Ford 1979). Knowledge of the internal defense capabilities of oysters is essential to the progress of these programs and understanding the environmental role in defense will aid in the breeding of disease-resistant stock and their movement to new localities. The study presented here was one in a series designed to outline the effects of the environment on cellular defense mechanisms.

Hemocytes of marine bivalve molluscs, as a consequence of their involvement in phagocytosis, encapsulation, inflammation, and wound plugging, are believed to make the greatest contribution to host defense against parasites and pathogens (Foley and Cheng 1975). Defense-related activities of hemocytes from American osyters (*Crassostrea virginica*) are known to be influenced by temperature, salinity, and habitat. Elevated temperatures have been observed to increase in vivo pinocytosis (Feng 1965), migratory activity and phagocytosis (Feng and Feng 1974), and in vitro rates of bacterial phagocytosis (Foley and Cheng 1975). Acute increases in salinity have been shown to retard hemocyte spreading and locomotion, and locomotion was retarded for low-salinity oysters even after a month of acclimation at high salinity (Fisher and Newell 1986a). Studies on the European flat oyster (*Ostrea edulis*) have shown a decrease of in vitro foreign-particle binding by hemocytes from oysters exposed 4–8 h to either increased temperature or decreased salinity (Fisher et al. 1987). Differences have been found between the hemocyte activities of *C. virginica* from a high-salinity oceanic habitat and from a low-salinity estuarine habitat (W. S. Fisher, unpubl. data; Fisher and Newell 1986a).

Binding of foreign particles by hemocytes has been employed by several investigators to assess the capacity of invertebrate cellular defense (Rowley and Ratcliffe 1979; Brehelin and Hoffman 1980; Fisher et al. 1987). It is a valuable measurement of hemocyte activity because it represents the culmination of several cell processes (e.g. spreading, locomotion, chemotaxis, and non-self-recognition) required in phagocytosis. Particles that bind to the surfaces of hemocytes will probably be phagocytosed (Pearsall and Weiser 1970), but binding can be distinguished from ingestion as a separate step in the phagocytic process (Rabinovitch 1967; Smith and Rommel 1977; Fisher 1986). Binding of foreign particles by oyster hemocytes may be the result of agglutinins (lectins) on the cell surfaces (Vasta et al. 1982; Cheng 1983).

This study measured the effects of the environment and habitat on in vitro cellular activities to determine optimal and minimal salinity–temperature combinations. The data positively correlate certain cell activity measurements with the more traditional methods of foreign-particle (bead) binding and support the association of these activities to defense-related functions of the hemocytes.

Materials and Methods

Oysters were collected from Wachapreague, Virginia (WP), an oceanic habitat, on 19 June 1985 (S = 33; 24°C) and from Tred Avon River, Maryland (TA), an estuarine portion of the Choptank River in the Chesapeake Bay, on 21 June 1985 (S = 13; 25°C). Seventy-two oysters from each habitat were marked and divided equally among six aquaria maintained at 22°C for 14 d while salinities were adjusted to 6, 18, or 30 ppt. The temperature for half the oysters at each salinity was then gradually raised over a 7-d period to 30°C. Thus, 144 total oysters from two habitats were distributed equally (12 replicates) into three salinities at two temperatures.

Hemocyte activity tests — After 23–24 d of acclimation at the various salinity and temperature combinations, the oyster shells were notched near the adductor muscle and hemolymph withdrawn with a syringe. Two drops of hemolymph were added to wells of a tissue culture plate containing 1 mL of artificial seawater at an iso-osmotic salinity (ASW) or at an osmotic concentration equivalent to a salinity increase of 12 (ASW + 12). For comparison with previous methodology (Fisher and Newell 1986a), a second hemocyte withdrawal was made from the same oysters within 15 min of the first. Hemocytes were diluted 1:1 with an iso-osmotic solution of calcium- and magnesium- free artificial seawater + 2% ethylenediaminetetraacetic acid, added to tissue culture plates as above, washed, and 1 mL of ASW or ASW + 12 added to initiate cell activity. Every 5 min after the addition of ASW, hemocytes were examined under an inverted microscope for ameboid shapes forming along the periphery of hemocyte aggregates. The time required for ameboid hemocytes to appear along the full periphery of the majority of aggregates was noted as the time to hemocyte spreading ("TTS" for cells in ASW, and "TTS + 12" in ASW + 12). Many of these procedures and all solution components are described in greater detail in Fisher and Newell (1986a).

Rate of hemocyte locomotion (ROL) was measured in vitro from both hemolymph withdrawals with the use of the inverted microscope. Selected hemocytes (15–20) were traced on a video monitor over a 5-min period. Only cells at iso-osmotic salinities (ASW) were measured and all measurements were made within 90 min of withdrawal. TTS, TTS + 12, and ROL measurements were all made at acclimation temperatures of 22 or 30°C.

Binding of latex beads — Foreign-particle binding was tested with 2-μm-diameter fluorescent latex beads (Fluoresbrite carboxylate microspheres; Polysciences, Inc., Warrington, PA 18976). Beads were diluted in ASW at S = 6, 18, and 30 (to test hemocytes acclimated to those salinities) to a final concentration of 2×10^5/mL. Beads formed some aggregates, but were diluted from the same stock. A single drop of undiluted

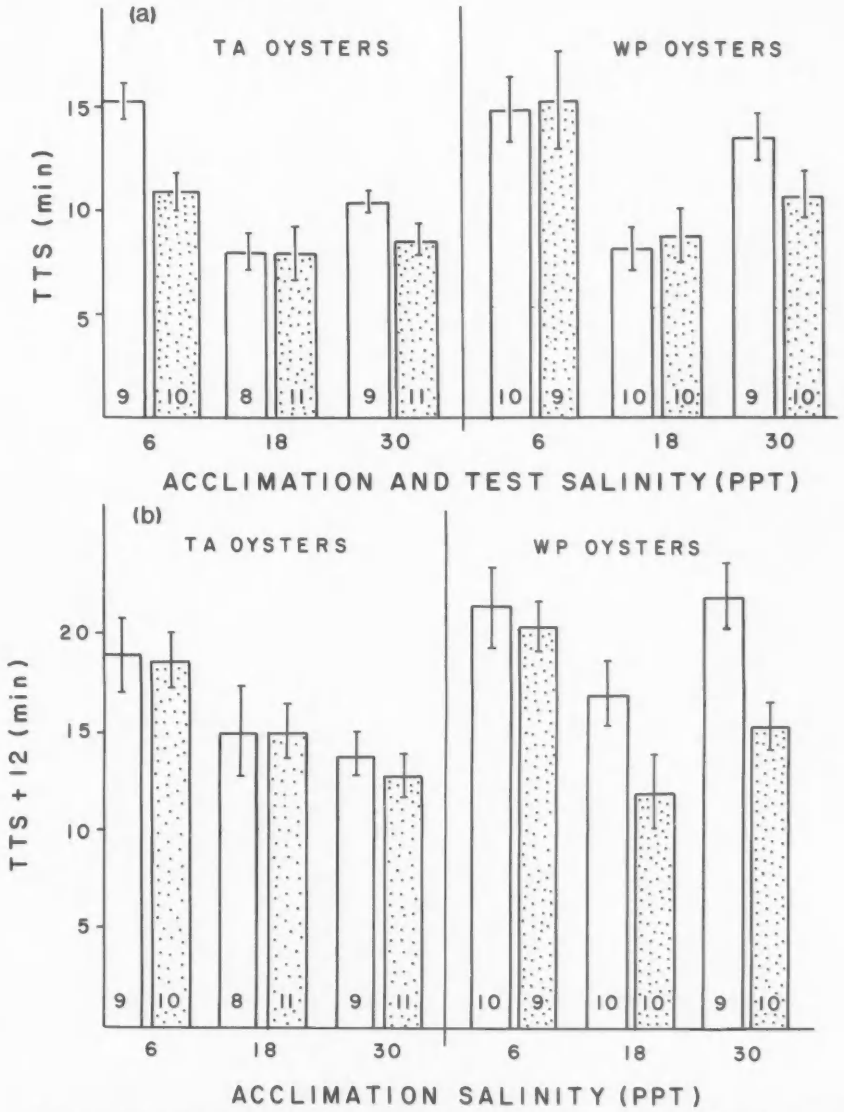

FIG. 1. (a) Time to hemocyte spreading (TTS) was measured for Tred Avon (TA) and Wachapreague (WP) oysters acclimated to three salinities (6, 18, or 30 ppt) and two water temperatures (22°C (open bars) or 30°C (stippled bars)). In vitro tests were conducted at acclimation salinities and temperatures. (b) TTS + 12 measurements for hemocytes from TA and WP oysters were made under the same conditions except that the in vitro test salinity was 12 higher than the acclimation salinity (i.e. S = 18, 30, or 42). Oyster sample size and standard errors are shown on the bars.

hemolymph was added to a slide and maintained in humid conditions at either 22 or 30°C. After 15 min to allow cell spreading, one drop of bead solution was added. After another 15 min, the slides were dipped five times in ASW to remove unbound beads and fixed in 10% formalin. Using an epifluorescent microscope, two randomly selected fields-of-view with 30–80 hemocytes were examined for the number of hemocytes, the number of hemocytes with bound beads, and the number of beads bound. From the averages of these counts, three indices were computed: percent adhesive hemocytes (%AH), beads per adhesive hemocyte (B/AH), and beads per total hemocyte count (B/TH).

Binding of bacteria — Three preliminary tests were conducted to compare the latex bead method with in vitro challenges of live bacteria. In two tests, bacteria (*Vibrio parahemolyticus*) grown on agar plates overnight were diluted in ASW at S = 12, 24, and 36 to a concentration of 10^6/mL. Hemocytes from TA oysters (S = 14) were withdrawn undiluted and allowed to spread on six slides for 15 min. Hemolymph was drawn off and the bacterial solutions at each salinity were added to duplicate slides.

TABLE 1. Correlation table of all parameters measured from undiluted withdrawals at all salinity and temperature regimes. The top number is the correlation value and the bottom number is the level of significance. These analyses were conducted by the Statistical Analysis System (SAS) at the University of Maryland. *Significance at 0.05 level; **significance at 0.01 level.

Parameter	*S*	%AH	B/AH	B/TH	ROL	TTS	TTS + 12
Temperature	0.041 0.666	0.323 0.001**	0.002 0.981	0.225 0.018*	0.300 0.001**	−0.208 0.029*	−0.239 0.011*
Salinity (*S*)	—	−0.130 0.173	0.087 0.362	−0.043 0.653	0.055 0.567	−0.270 0.004**	−0.245 0.010**
Percent adhesive hemocytes (%AH)		—	0.208 0.029*	0.785 0.001**	0.219 0.021*	−0.305 0.001**	−0.225 0.018*
Beads per adhesive hemocyte (B/AH)			—	0.620 0.001**	0.125 0.191	0.157 0.099	0.140 0.143
Beads per total hemocytes (B/TH)				—	0.288 0.002**	−0.124 0.194	−0.059 0.535
Rate of locomotion (ROL)					—	−0.051 0.596	0.011 0.908
Time to hemocyte spreading (TTS)						—	0.349 0.001**
Time to hemocyte spreading, acute salinity increase (TTS + 12)							—

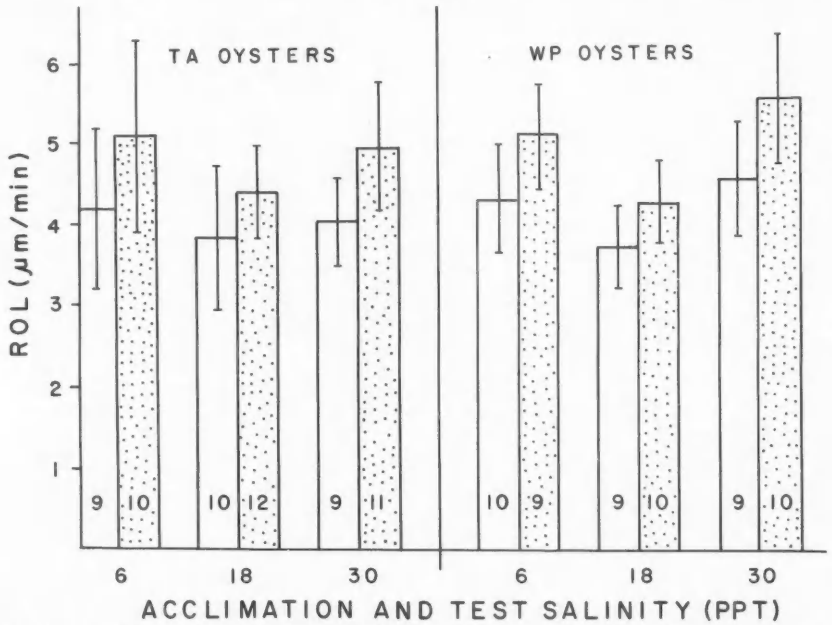

FIG. 2. Rate of hemocyte locomotion (ROL) for TA and WP oysters acclimated to and measured at three salinities (S = 6, 18, or 30) and two water temperatures (22°C (open bars) or 30°C (stippled bars)). Sample size and standard errors are shown on the bars.

After 15 min, the slides were dipped five times in iso-osmotic ASW to remove unbound bacteria, stained for 1 min in a 0.1% solution of acridine orange, and 25–50 randomly selected hemocytes on each slide examined under an epifluorescent microscope to determine the number of adhering bacteria (Smith and Rommel 1977). The same methodology was used in a third experiment with fluorescent latex beads replacing the bacteria.

Results

Hemocyte activity tests — Hemocytes generally spread faster at 30°C than at 22°C (Fig. 1a), but the difference was not significant by analysis of variance (ANOVA, $p > 0.05$). Overall, TTS was fastest at $S = 18$ and slowest at $S = 6$. A Student–Newman–Kuels (SNK) test showed these differences significant for TA-22°C, WP-22°C, and WP-30°C. TA hemocytes were faster than WP hemocytes ($p = 0.02$). Analysis by the Statistical Analysis System (SAS) at the University of Maryland showed that TTS was negatively correlated with temperature and salinity (Table 1).

With few exceptions, an acute in vitro increase in salinity (ASW + 12) resulted in longer cell spreading times (TTS + 12; Fig. 1b) compared with those tested at acclimation salinities (TTS; Fig. 1a). There were also significant differences due to habitat, salinity, and habitat–salinity interaction (ANOVA, $p < 0.01$). Overall, TTS + 12 response of hemocytes was faster at 30°C than at 22°C but significant only for WP hemocytes at acclimation salinities of 18 and 30. Hemocytes from both habitats were significantly slower in their TTS

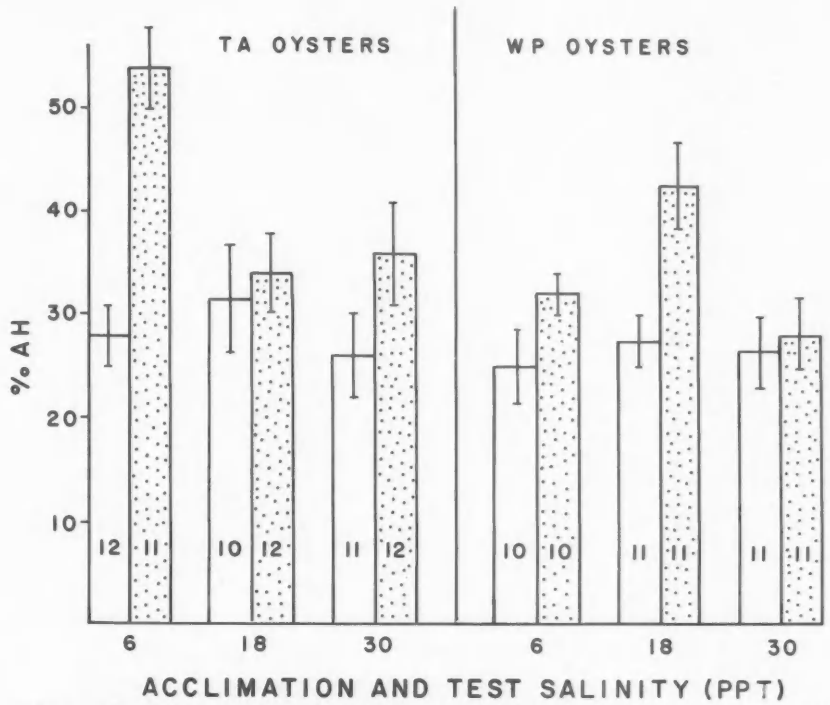

FIG. 3. Percent of adhesive hemocytes (%AH) for TA and WP oysters acclimated to and measured at three salinities (S = 6, 18, or 30) and two water temperatures, (22°C (open bars) or 30°C (stippled bars)). Standard errors and sample sizes are shown on the bars.

+ 12 response after acclimation at S = 6, and WP hemocytes responded faster after acclimation at S = 18 (SNK, $p < 0.01$). As with TTS measurements, TTS + 12 measurements revealed that TA hemocytes responded faster than WP hemocytes (SNK, $p < 0.01$). TTS + 12 measurements were positively correlated with TTS (SAS) and negatively correlated with salinity and temperature (Table 1).

ROL was significantly faster at 30°C for oysters of both habitats (Fig. 2). Hemocytes were slower at 18 ppt salinity (SNK, $p < 0.5$). ROL was positively correlated (SAS) with temperature (Table 1), but there were no significant correlations with TTS or TTS + 12 measurements.

Using SAS, comparison of the above results (from undiluted hemolymph withdrawals) with the EDTA-dilution technique withdrawals of Fisher and Newell (1986a) showed a positive correlation for TTS (0.382), TTS + 12 (0.400), and ROL (0.430) with very high significance ($p < 0.001$) in each case. However, both TTS and TTS + 12 measurements were faster in chelated withdrawals (ANOVA, $p < 0.01$).

Foreign-particle binding — There were highly significant (ANOVA, $p < 0.001$) differences in the number of hemocytes that bound latex beds (%AH) when analyzed by habitat, temperature, the interactions of habitat by salinity (two-way), and habitat by salinity by temperature (three-way). More beads were bound at 30°C than at 22°C in all cases (Fig. 3), but this was significant (SNK, $p < 0.05$) only for TA oysters at S = 6 and for WP oysters at S = 18. %AH was positively correlated (SAS) with temperature and ROL and negatively correlated with TTS and TTS + 12 (Table 1).

B/AH revealed no differences with temperature or habitat, but a significant decrease at S = 18 (not shown). Averages were 5.4, 3.6, and 5.1 B/AH for hemocytes at S = 30, 18, and 6, respectively.

B/TH showed significant differences for temperature and salinity. The highest number of beads were bound by TA hemocytes at S = 6 and 30°C (Fig. 4). B/TH measurements were positively correlated by SAS with B/AH, %AH, temperature, and ROL (Table 1). Negative correlations with TTS and TTS + 12 were not significant.

Binding of live bacteria decreased with acute salinity increases in two in vitro experiments (Table 2). This was demonstrated by the number of adhesive hemocytes, the number of bacteria per adhesive hemocyte, and the average number of bacteria for all hemocytes counted. The same pattern of results were obtained when latex beads were added to the cells instead of bacteria.

Discussion

Foreign particle binding by *C. virginica* hemocytes in vitro showed a positive (SAS) correlation with temperature and rate of hemocyte locomotion (Table 1, %AH and B/TH). Although a causal relationship has not been established, one interpretation is that warmer temperatures increased hemocyte locomotion which increased the number of hemocyte encounters with the beads. The negative correlation of TTS and TTS + 12 with %AH (Table 1) implied that hemocytes which spread quickly were also more able to capture foreign particles within the allotted time period.

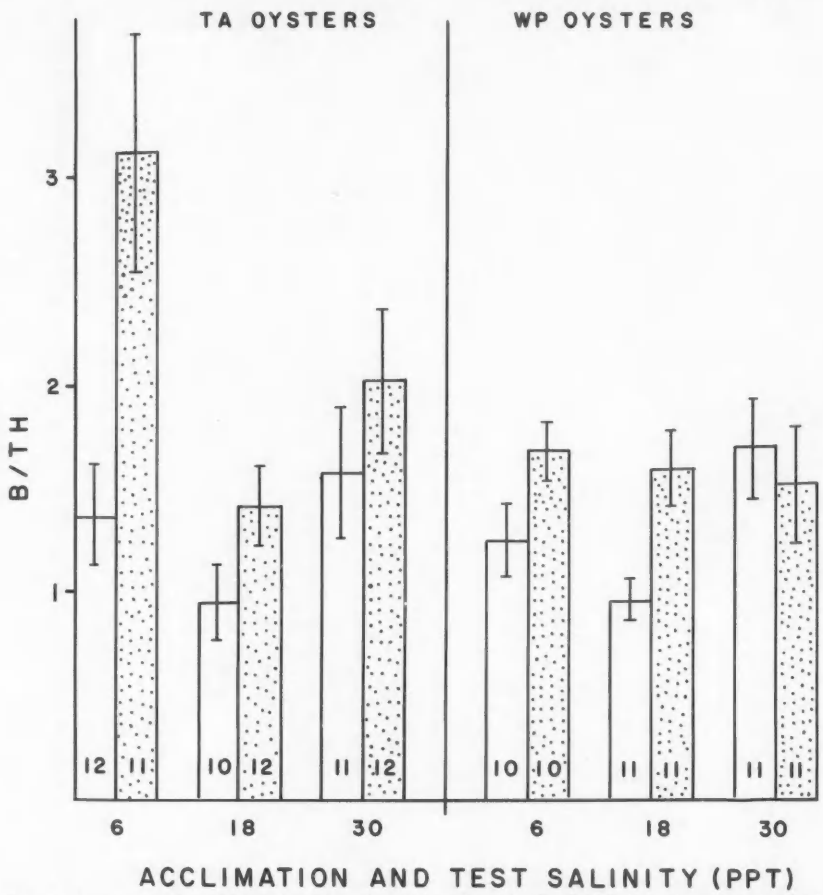

FIG. 4. Average number of beads bound by the total number of cells (B/TH) in a microscope field-of-view (30–80 cells) was measured for TA and WP oysters acclimated to three salinities (S = 6, 18, or 30) and two water temperatures, (22°C (open bars) or 30°C (stippled bars)). Standard error and sample sizes are shown on the bars.

TABLE 2. Comparison of hemocyte adhesion of *Vibrio parahemolyticus* (experiments 1 and 2) and latex beads (experiment 3) tested in vitro at three different salinities. Oysters were collected from and held at S = 13–14 prior to hemocyte withdrawal. %AH = percent adhesive hemocytes; B/AH = beads (or bacteria) per adhesive hemocyte; B/TH = beads (or bacteria) per total hemocytes.

	Vibrio parahemolyticus								
	Experiment 1			Experiment 2			Beads Experiment 3		
Salinity (ppt)	%AH	B/AH	B/TH	%AH	B/AH	B/TH	%AH	B/AH	B/TH
12	16.0	1.51	0.24	46.0	3.19	1.54	81.3	3.43	2.80
24	4.0	1.00	0.04	18.0	1.65	0.36	62.5	3.07	1.92
36	3.0	0.50	0.03	10.0	1.50	0.16	33.3	2.18	0.75

The retarded hemocyte spreading caused by an acute salinity increase (compare Fig. 1a and 1b) is in agreement with Fisher and Newell (1986a). This may indicate that osmotic balance is a precondition for hemocyte spreading. If so, then the difference between TTS and TTS + 12 measurements represents the time required for cells to regulate a salinity increase of 12. An acute salinity increase also retarded in vitro capture of *V. parahemolyticus* and latex beads (Table 2).

The lack of correlation between TTS measurements (Table 1) and ROL supports an earlier speculation (Fisher and Newell 1986a) that hemocyte spreading and locomotion involve separate cell processes. Locomotion is dependent on the ameboid shape, but is not necessarily influenced by the same factors that influence the cell's ability to reach an ameboid shape.

The high rate of foreign-particle binding exhibited by TA hemocytes at S = 6 and 30°C (Fig. 3 and 4) is an interesting

anomaly for two reasons: (1) low salinity retarded hemocyte spreading (Fig. 1a) even at 30°C and (2) ROL was not increased for the same samples. It is possible that the retarding effect on TTS was the only effect of low salinity on the hemocytes and that, once spread, low salinity did not influence other activities. Indeed, this is reflected in the equal ability of low-salinity oyster hemocytes to locomote (Fig. 2). The increased bead binding at $S = 6$ and 30°C did not appear to be a consequence of increased ROL, even though the correlation is well established in this study (Table 1). Perhaps cell surface receptors are able to recognize "non-self" particles under these conditions more than any other. Species differences in non-self-recognition have been noted using similar techniques (Fisher 1988), but potential environmental influences are unknown.

The results from the chelated withdrawal were similar to the undiluted withdrawal except for relatively faster TTS and TTS + 12. Thus, in this study at least, the chelated techniques did not alter the relative hemocyte activities or their response to environmental changes.

Estuarine oysters appear to have the greatest variability in their acclimated response to environmental factors. In a previous study (Fisher and Newell 1986a), TTS was retarded by acute increases in salinity but, after a month of acclimation, was rapid at all salinities tested ($S = 6$–36). ROL was retarded by acute increases in salinity and this effect was sustained in estuarine oysters even after a month of acclimation. Estuarine oysters in this study, after 3 wk of acclimation, showed a retarded TTS at the lowest salinity (Fig. 1a) and no salinity effect on ROL (Fig. 2).

These results indicate that estuarine oyster hemocyte activity may be influenced by a complex of factors, including environmental history and seasonality as well as temperature and salinity. TA oysters were collected from the same site, but in the previous study (Fisher and Newell 1986a) were collected in May 1984, a year of heavy rainfall in Chesapeake Bay which caused reduced salinities ($S = 6$–8) and little or no spawning by the TA oysters. Oysters in the present study were collected in June 1985, a year of high salinities ($S = 13$–14) and exceptionally high spawning. It is possible that prior exposure to higher salinities in this study (1985) played a role in hemocyte ability to spread and locomote more quickly at high test salinities and less quickly at low test salinities. The effect of spawning is not known, but protein and carbohydrate levels from TA oyster hemolymph showed a dramatic decrease at the time of spawning in 1985 (Fisher and Newell 1986b).

In contrast, oceanic oysters showed acute and acclimated hemocyte responses in general agreement with previous (Fisher and Newell 1986a) results. Less environmental variability in the WP habitat may contribute to the greater consistency in hemocyte responses on a yearly basis. Further monitoring of TA and WP oyster hemocyte responses will be necessary to clarify the influence of environmental conditions and seasonality.

This study demonstrates that in vitro hemocyte activities, such as hemocyte spreading, locomotion, and foreign-particle binding, are affected by the environment. It also demonstrates that TTS and ROL measure different cell mechanisms, but are significantly related to foreign-particle binding, a known defensive function. Although measurements of in vitro hemocyte activities can serve only as indicators for in vivo and in situ conditions, they may eventually provide a better understanding of environmental effects on oyster defense mechanisms. Such an understanding is vital to selective breeding programs for disease-resistant oysters, whether differences are heritable or environmental, and whether disease resistant characteristics are applicable over a range of environments.

Acknowledgements

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Poststocking Movements of Large Coho Salmon (*Oncorhynchus kisutch*) Fingerlings Introduced in February into a Small Coastal Stream

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Irvine, J. R. 1988. Poststocking movements of large coho salmon (*Oncorhynchus kisutch*) fingerlings introduced in February into a small coastal stream. Can. J. Fish. Aquat. Sci. 45: 1316–1321.

Juvenile coho salmon (*Oncorhynchus kisutch*), introduced into alternating 100-m sections of the lower kilometre of a steep-gradient stream in British Columbia, dispersed rapidly along the stream. After several days, fish originating from downstream of unstocked sections were almost as numerous in the unstocked sections as fish originating from upstream. The largest coho ($\bar{w}$ = 110 g), many of which had undergone smoltification, left the stream almost immediately after stocking. Six weeks after stocking, virtually no coho remained in the stream. Freshets apparently were responsible for displacing many of the fish out of the system.

De jeunes saumons cohos (*Oncorhynchus kisutch*), introduits dans des tronçons de 100 m alternants du dernier kilomètre d'un cours d'eau à forte pente en Colombie-Britannique, se sont dispersés rapidement le long du cours d'eau. Après plusieurs jours, les poissons provenant de la partie située en aval des tronçons non ensemencés étaient presque aussi nombreux dans ces tronçons que les poissons provenant de la partie située en amont. Les plus gros saumons cohos ($\bar{w}$ = 110 g), dont un grand nombre avait subi la smoltification, ont quitté le cours d'eau presque immédiatement après l'ensemencement. Six semaines après l'ensemencement, il ne restait pratiquement plus de saumons cohos dans le cours d'eau. Des crues nivales ont apparemment été responsables de la sortie d'un grand nombre des poissons du réseau hydrographique.

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The stocking of juvenile salmonids into streams, in particular coho salmon (*Oncorhynchus kisutch*), is a common enhancement strategy in the Pacific Northwest (Bilby and Bisson 1987; Nickelson et al. 1986). In British Columbia, underyearling coho less than 5 g are often introduced with the expectation that these fish will live and grow in the stream until the following spring (outplanting, R. Bams, Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C., pers. comm.). In other cases, larger juvenile salmon classified as smolts are introduced, but these are not expected to remain in freshwater for a significant period of time. Scatterplanting of underyearlings is usually recommended, rather than point releases, as it is felt that by distributing fish over as much of a water course as possible, survival is improved (Fedorenko and Shepherd 1986). However, little work has been conducted on the effectiveness of different stocking techniques, or on the instream dispersal of fish following stocking (Reisenbichler and McIntyre 1986). This note reports observations of juvenile coho salmon movements within a stream following their stocking as smolts.

Methods

Cypress Creek, near the West Vancouver Laboratory in southwestern British Columbia (Fig. 1), is a steep-gradient stream which varies in discharge from 0.05 m^3/s during dry summer periods to greater than 50 m^3/s following heavy winter storms. Historically, Cypress Creek had a significant run of coho salmon, but the run apparently became extinct in the 1970's. Various attempts to reestablish the run have been made, and in the autumn of 1986, at least 23 coho returned to the creek to spawn (M. Waldichuk, Department of Fisheries and Oceans, West Vancouver Laboratory, West Vancouver, B.C., pers. comm.).

The lower creek was divided into ten 100-m long sections with the downstream section beginning at the confluence of the creek mouth and the ocean at low tide and the upper section terminating near a waterfall impassable to upstream migrating fish (Fig. 1). A small dam in Section 7 is a barrier to the upstream movement of juvenile salmonids, but adults can successfully pass.

In the fall of 1985, eggs were obtained from fish at the Capilano Hatchery on the nearby Capilano River. The eggs were incubated at the West Vancouver Laboratory in well water, and commencing in May 1986, the resulting fry were raised in water diverted from Cypress Creek. Growth of most fish during the summer and fall was rapid, and in November, coded wire tags were inserted in those fish longer than 10 cm. At this time, all fish longer than 19 cm were separated (Group 1), and approximately the same number of fish between 10 and 19 cm were randomly selected (Group 2) as a comparison (Table 1). Many of the fish in Group 1 were silver and a few of the males were

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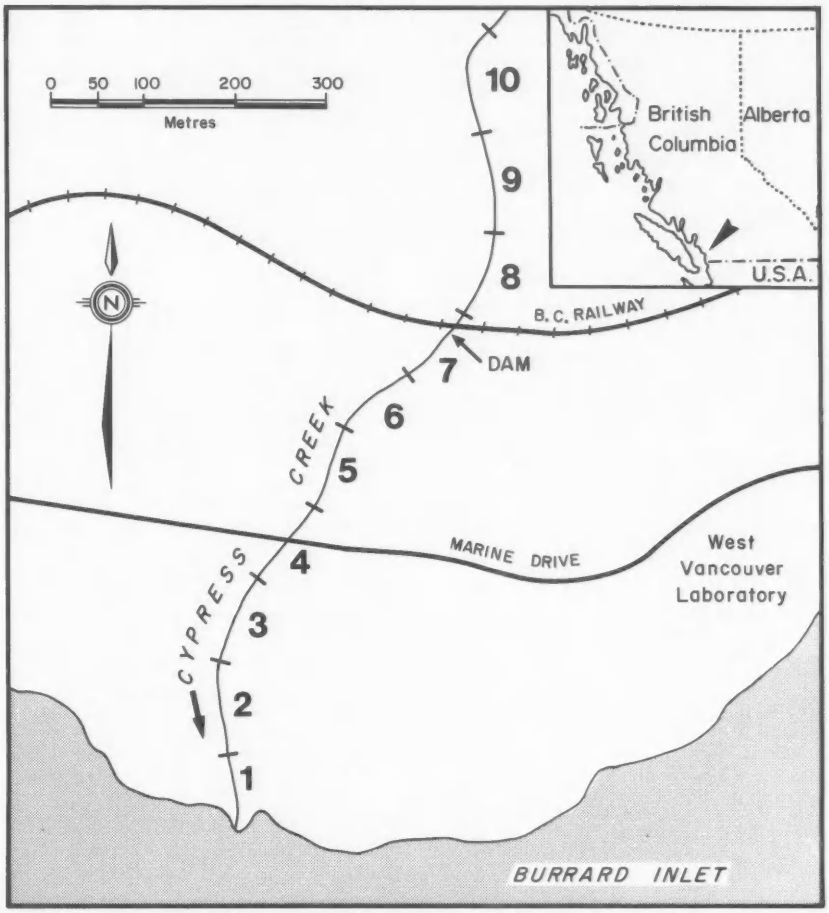

FIG. 1. Lower Cypress Creek illustrating the study sections. Inset shows location of study area in southwestern British Columbia.

TABLE 1. Mean fork lengths (cm) and weights (g) (±95% confidence intervals in parentheses) of the various groups (1–8) of coho introduced to Cypress Creek, the numbers stocked, and the stream sections the groups were introduced to.

	1	2	3	4	5	6	7	8
Length	21.7 (2.8)	14.3 (4.3)	14.5 (4.0)	15.3 (5.2)	14.4 (3.7)	15.5 (13.2)	14.7 (4.0)	14.3 (4.2)
Weight	109.8 (35.2)	36.2 (36.4)	37.2 (30.7)	44.7 (44.2)	36.3 (29.4)	39.0 (30.1)	39.5 (33.5)	36.8 (32.6)
Number stocked	559	584	400	410	431	367	395	402
Section(s)	8 and lower portion of 9		6		4		2	

sexually mature. Those fish between 10 and 19 cm remaining after selection of Group 2 were randomly divided into six groups. In early December, all the fish were cold-branded (Mighell 1969). Five different brands were used, with fish branded differently from the following groups: 1; 2; 3 and 4; 5 and 6; and 7 and 8. A minimum of 50 fish from each group were then weighed and measured. The fish were held until 2 February 1987 to monitor mortality due to handling, and on that date, they were introduced into Sections 2, 4, 6, 8, and the lower part of 9, as indicated in Table 1. No fish were stocked

in Section 1, 3, 5, 7, or 10. Within the stocked sections, approximately four fish per linear metre were introduced along each bank of the creek. The mean creek width at the time of stocking was 7.7 m and the stocking density in the sections stocked was 1.02 fish/m^2.

Near the centre of each of Sections 2–8, one 20-m length of stream judged to be representative of its section was selected for population surveys. A 1.75-m-wide strip along one or both sides of these subsections was electrofished during 18–19 December (prestocking), 3–6 February (immediately post-stocking), and on 16 March. On 3 February, Sections 2–5 were electrofished, but late in the afternoon a rainstorm began which continued into the next day. Rain and a minor freshet on 4 February made electrofishing the creek impossible but did enable a comparison to be made between pre- (3 February) and postfreshet (5 February) in sections that had been electrofished on 3 February. Usually, three passes along each bank within each subsection were made, always in an upstream direction with one person carrying an electrofishing anode and two people carrying dipnets. Successive passes generally alternated between the two sides of the creek to give uncaptured fish the opportunity to recover before electrofishing there again and thereby reduce the likelihood of the probability of capture varying between runs. While we were electrofishing, we occasionally observed fish moving from one side of the creek to the other, but we never saw fish moving between stream sections. All coho were examined for cold-brands and released along with other fish captured after all electrofishing was completed. Fish were released at the approximate location within the creek from where they were captured. Electrofishing results were analysed using the sequential removal method of Zippen (1956) to obtain population estimates with associated 95% confidence intervals. In some cases, additional electrofishing was carried out to increase the sample size of cold-branded coho.

A 15-m beach seine was used to sample fish at the mouth of Cypress Creek (Section 1). At least eight sweeps were made on each of the following days: 18 December, 3 February and 5 February.

Results

Juvenile coho salmon were virtually absent from Cypress Creek during the December prerelease electrofishing survey

TABLE 2. Number of coho and trout per square metre (±95% confidence intervals in parentheses) along the left (L) and right (R) banks of Cypress Creek. Coho densities on 2 February are the stocking densities which were calculated assuming uniform distribution across the stream; as fish appeared to be clumped along each bank, these densities are underestimates of the densities along each bank. When no number is given, no density estimate was made; min. is the minimum density (confidence intervals could not be estimated).

		18 Dec.		2 Feb.		3 Feb.		5–6 Feb.		16 Mar.	
Section	Bank	Coho	Trout	Coho	Trout	Coho	Trout	Coho	Trout	Coho	Trout
2	L	0.03 min.	0.14 (0.01)	1.02		1.50 (0.18)	0.09 min.	1.11 (0.01)	0	0	0
	R	0	0.03 min.	1.02		0.67 (0.05)	0.06 min.			0.03 min.	0.03 min.
3	L	0	0.06 (0.04)	0		1.48 (0.06)	0	0.83 (0.02)	0	0	0
	R	0	0.09 (0.02)	0		0.95 (0.03)	0.03 min.			0.03 min.	0
4	L	0	0.06 min.	1.02		0.41 (0.64)	0			0	0
	R	0	0.14 (0.01)	1.02		3.66 (0.06)	0.14 (0.01)	0.69 (0.01)	0	0	0.13 (0.06)
5	L	0	0.34 (0.01)	0		0.14 (0.01)	0.17 (0.01)	0.13 (0.06)	0.03 min.	0	0
	R	0	0.18 (0.03)	0		0.35 (0.03)	0.11 (0.15)			0	0.09 (0.02)
6	L	0	0.21 (0.06)	1.02				0.26 min.	0	0	0
	R	0	0.09 (0.02)	1.02				0.26 min.	0	0	0
7	L	0	0.09 (0.02)	0				0.14 min.	0	0	0.03 min.
	R	0	0.12 (0.02)	0				0.52 (0.04)	0.15 (0.04)	0	0
8	L	0	0.29 (0.13)	1.02				0.33 (0.26)	0	0	0.06 min.
	R	0	0.27 (0.07)	1.02				0.35 (0.26)	0.13 (0.06)	0	0.06 min.
All	Both	<0.001	0.15	0.58		1.15	0.09	0.46	0.03	<0.001	0.03

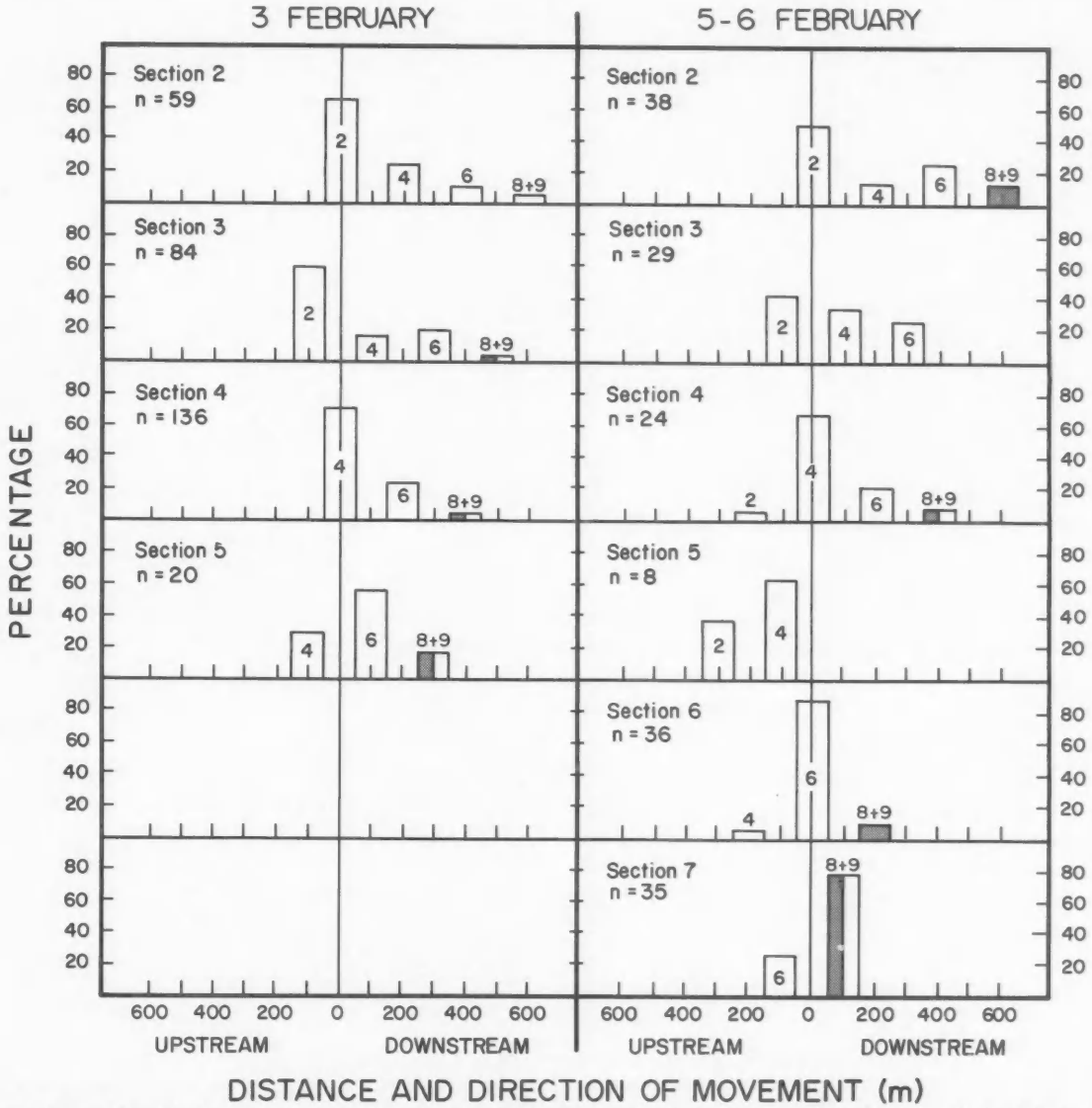

FIG. 2. Composition of catches of stocked coho in each section before (left panel) and after (right panel) the 4 February freshet. The section electrofished and the number of coho captured in it are given in the upper left corner of each graph. The numbers in or above the columns indicate the section of stocking origin for the recaptured fish. The proportions of the total number of fish captured at each location and date originating from different stocking locations are given as percentages. Distances and direction of movement from the point of release are indicated on the abscissa. Columns on the left-hand side of the central vertical line in each panel represent fish that swam upstream and columns on the right-hand side represent fish that swam downstream to their point of capture. Columns bisected by the vertical line represent fish that did not move out of the section in which they were introduced. The proportions of the total number of recaptured fish originating in Sections 8 and 9 that were of the smaller (normal) size group are depicted by the shaded portion of the Sections 8 and 9 columns.

(Table 2). The day after the release (3 February), densities were high in the five sections that were sampled, higher than the mean stocking density, indicating that few of the fish introduced to the lower creek had left the stream. Significant numbers of coho were caught on 3 February in unstocked sections (3 and 5), which confirmed that considerable instream movement had occurred shortly after the release. Coho densities at all sites resampled were less after the freshet on 4 February than before, significantly so at most sites (Table 2), indicating noticeable movement from the stream over a 3- to 4-d span. Coho densities in February were generally higher in stocked sections than adjacent unstocked sections and were highest in those sections furthest downstream. When the creek was next sampled on 16 March, virtually no coho were caught. Beach seining at the mouth of the creek failed to capture any branded coho although other fish were caught.

TABLE 3. Numbers of branded coho caught in each section of Cypress Creek electrofished during 3, 5, and 6 February partitioned according to the section they were introduced to.

Section stocked	Section of capture							
	2	3	4	5	6	7	8 + 9	Σ
2	56	62	1	3	0	0	0	122
4	18	25	113	11	2	0	0	169
6	15	23	35	11	31	8	0	123
8 + 9	8	3	11	3	3	27	57	112
Σ	97	113	160	28	36	35	57	526

Trout (*Salmo gairdneri*) densities declined significantly between December and February–March (Table 2), but this was not necessarily a consequence of the introduction of coho. The trout captured comprised at least three age classes.

For sections 2, 3, and 4, the proportions of fish captured from the various stocking locations (Fig. 2) before and after the 4 February freshet were not significantly different (contingency table chi-square tests, $p > 0.05$), but they were different in Section 5 ($p < 0.005$). On 3 February the proportion of fish captured that had swum upstream to Section 5 was smaller than expected from chi-square tests. On 5 February the situation was reversed.

In Section 2, 56 coho (58%) of those captured 3–6 February had been introduced to that section and 41 coho (42%) originated from upstream (Table 3). Of the 196 total captured in Sections 4 and 6, 144 coho (73%) had been introduced to these sections, 3 coho (2%) had moved upstream through an unstocked section, and 49 coho (25%) had moved downstream. Of the 176 coho captured in the unstocked sections (3, 5, and 7), 78 coho (44%) had moved upstream and 98 coho (56%) downstream. All of the coho captured in Section 8 originated from Sections 8 and 9 because of the barrier to upstream fish movement in Section 7. Because sampling effort was not the same in each section, detailed comparisons between numbers of captured fish in the different sections should not be made. However, it is worth noting that over one half of the captured fish introduced to Section 2 were caught upstream of Section 2, approximately two thirds of the captured fish from Section 4 were caught in Section 4, and two thirds of the captured fish from Section 6 were caught downstream of Section 6.

Many of the group of large coho stocked in Sections 8 and 9 appeared to leave the creek very rapidly, probably during the first night. On 3 February, 18 coho that had been introduced to Sections 8 and 9 were captured in Sections 2–5, and of these, 11 coho (61%) were of the smaller size group. During 5–6 February, 37 fish introduced to Sections 8 and 9 were captured below this area and 33 (89%) of them were from the smallest size category. When Section 8 and the lower portion of Section 9 were electrofished on 6 February, 57 coho were captured of which 52 (91%) were from the smaller size category.

Discussion

Few of the stocked coho appeared to leave Cypress Creek during their first 24 h in the stream with the exception of the large size group of fish. Many of these larger fish were silver in colour, and therefore presumably had undergone smoltification (Folmar and Dickhoff 1980); they probably migrated directly to sea. Since branded coho were not captured at the mouth of the creek when it was beach seined, the fish may have migrated away from the immediate vicinity of Cypress Creek. The freshet on 4 February apparently was responsible for displacing some of the fish out of Cypress Creek. A series of freshets occurring between the February and March population surveys may have displaced the remainder of the coho. Bilby and Bisson (1987) found that changes in discharge were responsible for triggering downstream movements of coho fry stocked in two streams in Washington.

A significant and rapid redistribution of coho within Cypress Creek occurred following stocking. Fish introduced to the upper sections quickly distributed themselves along the length of the stream, while many of the fish introduced to the lowest section stocked, Section 2, moved upstream, a few as far as Section 5. Unstocked sections of Cypress Creek had densities approaching those of stocked sections within several days of the release. This redistribution is contrary to what some workers have reported. Egglishaw and Shackley (1980) and Mortensen (1977) reported very little fish movement after having stocked eggs and unfed fry of Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*). Hume and Parkinson (1987) found that median dispersal distances for point-released steelhead (*S. gairdneri*) fry were generally <100 m over the summer. The rapid dispersal of coho within Cypress Creek was probably governed by the poor quality of habitat for young coho in the creek. Bilby and Bisson (1987) predicted that when pools and cover are rare in a stream, few juvenile coho will remain in the vicinity of where they are released. Other factors including the relatively large size of the fish stocked, their aggressive behaviour (Chapman 1962), and perhaps the time of year may have influenced their rapid dispersal. Both the size of coho and timing of their release were unusual in this experiment; Bilton et al. (1984) and Fedorenko and Shepherd (1986) recommended releasing 15- to 25-g coho smolts in June. Caution therefore must be used in interpreting this study. Nevertheless, it appears that when large juvenile coho are stocked in poor-quality habitat, instream dispersal can be significant. Therefore, point releases may be as successful a technique as scatterplanting under these circumstances.

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Retinal Structure of Sympatric and Allopatric Populations of Cutthroat Trout (*Salmo clarki clarki*) and Dolly Varden Char (*Salvelinus malma*) in Relation to their Spatial Distribution

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Henderson, M. A., and T. G. Northcote. 1988. Retinal structure of sympatric and allopatric populations of cutthroat trout (*Salmo clarki clarki*) and Dolly Varden char (*Salvelinus malma*) in relation to their spatial distribution. Can. J. Fish. Aquat. Sci. 1321–1326.

Retinal structures of cutthroat trout (*Salmo clarki clarki*) and Dolly Varden char (*Salvelinus malma*) were compared to determine if these features were consistent with the different light regimes in which the species live, depending on their sympatry or allopatry in lakes. Cone cell density in sympatric trout was over twice that of sympatric char and rod cell density was slightly less. The cross-sectional size of cone cells in sympatric trout was approximately 30% less than in sympatric char. The ratios of photoreceptor cells to ganglion cells in sympatric trout and char retinas were approximately 40:1 and 80:1, respectively. There were no differences between sympatric and allopatric trout with respect to these structural features. Estimates of cone cell density, cone size, and number of photoreceptor cells per ganglion cell for allopatric char were significantly different and intermediate between those for sympatric trout and char. All four populations had similar cone cell mosaics with four paired cones surrounding each single cone. Results are considered in relation to differences in vertical distribution and feeding of sympatric and allopatric populations in three study lakes.

On compare les structures rétiniennes de la truite fardée (*Salmo clarki clarki*) et de la Dolly Varden (*Salvelinus malma*) pour déterminer si ces caractéristiques correspondent aux différentes conditions de luminosité du milieu lacustre où vivent ces espèces selon qu'elles soient sympatriques ou allopatriques. La densité des cellules à cônes chez la truite sympatrique est plus de deux fois supérieure à celle de la Dolly Varden sympatrique tandis que la densité des cellules à bâtonnets est légèrement inférieure. La taille en coupe transversale des cellules à cônes de la truite sympatrique est d'environ 30 % inférieure à celle observée chez la Dolly Varden sympatrique. Les rapports des cellules photoréceptrices aux cellules ganglionnaires au niveau de la rétine de la truite et de la Dolly Varden sympatriques sont d'environ 40:1 et 80:1 respectivement. Il n'y a aucune différence entre la truite sympatrique et allopatrique en ce qui concerne ces caractéristiques structurales. Les estimations de la densité des cellules à cônes, de la taille des cônes et du nombre de cellules photoréceptrices par cellule ganglionnaire pour la Dolly Varden allopatrique sont significativement différentes et se situent à un niveau intermédiaire de celles s'appliquant à la truite et à la Dolly Varden sympatriques. Les quatre populations possèdent des mosaïques de cellules à cônes semblables, soit quatre cônes appariés entourant chaque cône individuel. On examine les résultats obtenus en fonction des différences observées dans la répartition verticale et l'alimentation des populations sympatriques et allopatriques dans trois lacs expérimentaux.

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Cutthroat trout (*Salmo clarki clarki*; hereafter referred to as trout) and Dolly Varden char (*Salvelinus malma*; hereafter referred to as char) are sympatric in many coastal lakes of British Columbia (Andrusak and Northcote 1970, 1971) as well as elsewhere in the Pacific northwestern region. In these areas, trout inhabit near-surface and onshore waters feeding mainly on zooplankton and surface insects whereas char concentrate in deeper, near-bottom waters feeding chiefly on benthic invertebrates and less on zooplankton. In some lakes these species are allopatric and in such situations utilize a greater depth and food range, taking surface, midwater, and benthic invertebrates (Andrusak and Northcote 1971; Hume and Northcote 1985; Hindar et al. 1988).

Since there are adaptive differences in retinal structure in species which commonly inhabit high irradiance regimes of near-surface waters compared with those which usually occur in areas of low irradiance in near-bottom waters (Anctil 1969; Ali and Anctil 1977), one might expect to find such differences in populations of trout and char living sympatrically or allopatrically.

Laboratory experiments examining the feeding behaviour of trout and char (Schutz and Northcote 1972) suggested that there might be differences in visual capabilities which affected their foraging success under low-irradiance regimes. This possibility has now been confirmed by Henderson and Northcote (1985) who demonstrated that the visual irradiance threshold and the saturation irradiance threshold of char were respectively about one and two orders of magnitude lower than those of trout. No detailed study of the retinal structure of either trout or char exists, although retinal features of several related species have been described (Ali 1959; Ali and Anctil 1976; Ali and Wagner 1980; Ali and Klyne 1984). Our purpose in this note is to (1) report some features of retinal structure which may underly functional differences in visual capability of sympatric trout and char, (2) determine if formerly sympatric populations after several generations in allopatry may have diverged in retinal structure, and (3) consider how differences in retinal structure may be related to lake distribution and feeding. At the outset it should be noted that our study in no way attempts to provide a complete comparison of retinal anatomy for these species nor to infer from the limited retinal features examined a complete functional model of visual capabilities in relation to behaviour. Such inferences would require information on other structural features not examined in our study and also on the characteristics of retinal photopigments which are known to vary in fish species inhabiting different environments (Muntz 1974). Nevertheless, the structural features we did examine have been used to study behavioural light-related responses in several other species of freshwater fishes (Ali and Anctil 1977; Ali et al. 1977; Ryder 1977).

Materials and Methods

Adult sympatric trout (19.2–20.8 cm fork length) and char (19.7–21.4 cm) used in histological examination of retinal structure were collected from Loon Lake in the University of British Columbia Research Forest. Adult allopatric trout (18.0–19.6 cm) and char (18.5–20.7 cm) were obtained from Placid and Dickson lakes, respectively. General features of these lakes have been described previously (Andrusak and Northcote 1971; Northcote and Clarotto 1975). Fish of the size range used would be maturing or mature adults and at least 3 yr old (Jonsson et al. 1984; Andrusak 1969). Ten animals of each species were collected from each lake and transported alive to the University of British Columbia where they were held at 10°C on a 16-h light/8-h dark irradiance regime for 7 d. At the end of 1 wk, each animal was beheaded while in a light-adapted state and the right eye enucleated. The cornea and lens of each eye were removed and the whole eye cup was fixed at room temperature in Bouin's solution for 48 h. The eye cup was then transferred to 70% ethanol and held for further processing.

The irradiance level used during the entire 16-h light period was approximately 6.6×10^{18} photons·m^{-2}·s^{-1} at the water surface. Irradiance was measured with a LI-COR quantum meter (model 185A) and attached underwater sensor. The sensor detected all radiation between 300 and 700 nm.

Prior to embedding, a 1-mm^2 section of material lying at the intersection of the dorsal–ventral and temporal–nasal axes (i.e. the central portion) of the eye cup, but excluding the ora serrata, was dissected out and dehydrated. This tissue, containing the retina, was then embedded in Spurr's medium. One half micrometre transverse and tangential sections were cut on a Reichert OM U3 ultramicrotome. Sections were stained in methylene blue.

Ten counts (250× magnification) of the densities of rod, cone, bipolar, and ganglion cells were taken from tangential sections (Ali and Anctil 1976) of retinal tissue of each fish. This resulted in a total of 100 counts of each cell type for each combination of sympatric and allopatric trout and char. All counts were based on the number of cells lying within the boundaries of a micrometre field. The area of the micrometre field was the same for all counts. Rod and cone cells were counted at the outer (external) nuclear layer, bipolar cells at the inner (internal) nuclear layer, and ganglion cells at the ganglion cell layer (see Ali and Anctil 1976). Measurements of the diameter of single cones and the longest and shortest axes of elliptically shaped paired cones were made in the region of the ellipsoid segments. Ten cones of each type were measured in each retina.

Comparison of the means of cell counts and measurements was accomplished using one-way analysis of variance. All statistical tests were one-tailed.

Results

The retinas of trout and char are qualitatively very similar; only that of the trout will be described in detail (Fig. 1). The retina consists of eight different layers (Fig. 1A) with an external limiting membrane between the visual cell layer and the outer nuclear layer. The rods and cones (Fig. 1B), located in the visual cell layer, are connected to the second-order neurones, the bipolar cells found in the inner nuclear layer. Ganglion cell dendrites make synaptic contact with bipolar axons in the inner plexiform layer, while axons of the ganglion cells converge to form the optic tract.

Two morphological types of cones were identified in the retinas of trout and char. Four paired cones, elliptical in cross-section, surrounded each of the single cones which were circular in cross-section (Fig. 1C).

The density of visual cells in the central retina of trout and char was high (Table 1). In both species, rods outnumbered cones by more than one order of magnitude. Cone density in the central retina of sympatric trout was higher ($P < 0.05$) than cone density in sympatric char by a ratio of approximately 2:1. Although cone density in the population of sympatric trout was slightly higher than the density found in allopatric trout, the

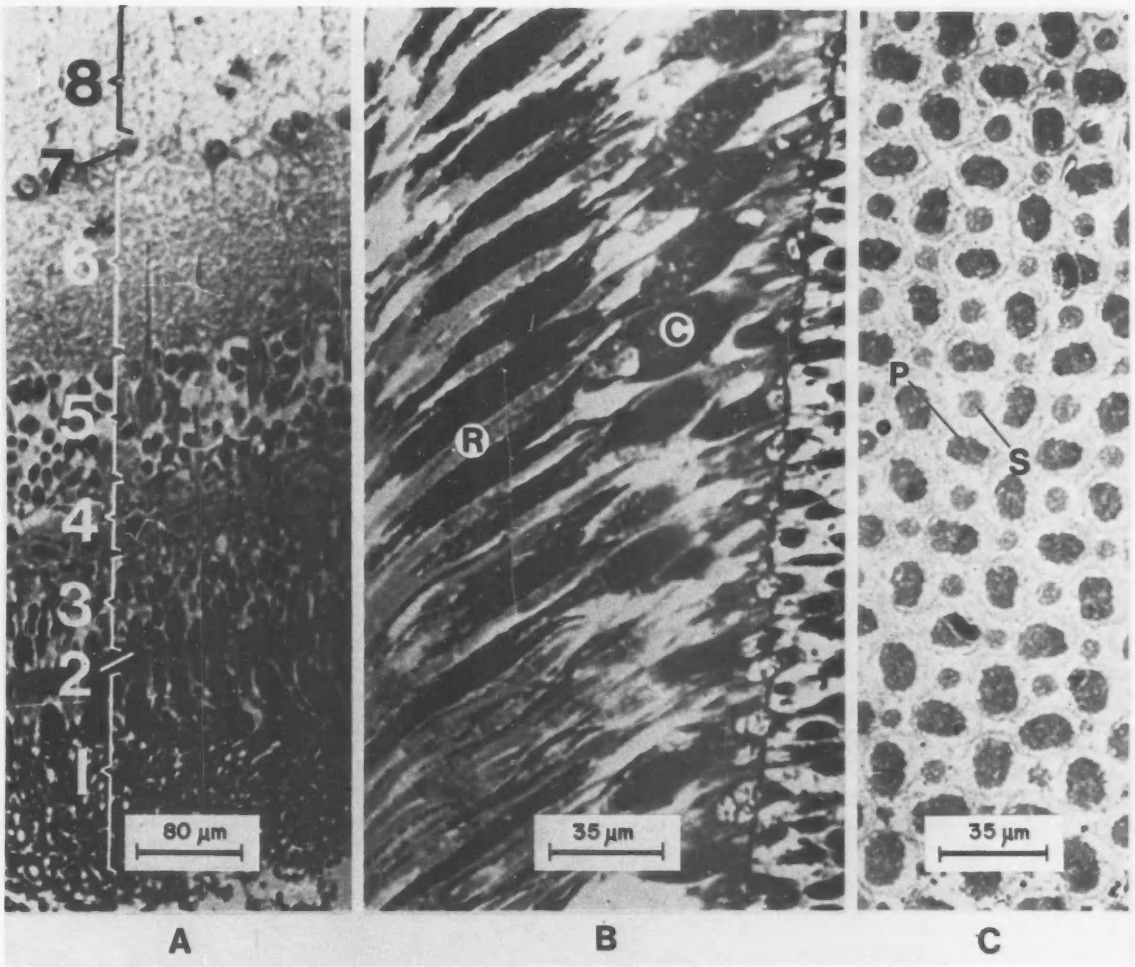

FIG. 1. Retinal structure of an adult cutthroat trout from Loon Lake, British Columbia. (A) Transverse section of the retina: 1, visual cell layer (containing epithelial pigment); 2, external limiting membrane; 3, outer nuclear layer; 4, external plexiform layer; 5, inner nuclear layer; 6, internal plexiform layer; 7, ganglion cell layer; 8, nerve fibre layer. ×330. (B) Transverse section through the visual cell layer of the retina; C, cone; R, rod. ×500. (C) Tangential section through the visual cell layer of the retina; S, single cone cell; P, paired cone cell. ×500.

TABLE 1. Comparison of the density (±95% CL) of rod and cone cells in the retinas of sympatric and allopatric cutthroat trout and Dolly Varden char.

Species	Density (1.0 mm^{-2}) Rod	Cone	Rod:cone ratio
Sympatric (Loon Lake)			
Trout	116 760 ± 2836	8840 ± 959	13.2:1
Char	129 687 ± 3001	3771 ± 750	34.4:1
Allopatric			
Trout (Placid Lake)	118 011 ± 2959	8340 ± 792	14.1:1
Char (Dickson Lake)	126 768 ± 2669	6005 ± 709	21.1:1

difference was not statistically significant ($P > 0.05$). However, a comparison of cone density between allopatric and sympatric char revealed a significantly ($P < 0.05$) higher density in the allopatric form.

Rod density in the retina of sympatric trout was significantly lower ($P < 0.05$) than the density found in sympatric char, opposite to the trend noted above for cone density in these two populations (Table 1). There was no significant difference ($P > 0.05$) in rod density between populations of sympatric and allopatric trout or sympatric and allopatric char.

Differences in cone size existed among the four populations of fish examined. Single cones in the retinas of sympatric trout were approximately 40.0% smaller ($P < 0.05$) than those of sympatric char (Table 2). No significant difference ($P > 0.05$) existed in the size of the single cones in sympatric and allopatric trout. Single cone cells of sympatric char were about 22% larger than those in the allopatric population (difference significant, $P < 0.05$). Sympatric trout had smaller paired cones (both axes significantly different, $p < 0.05$) than did sympatric char (Table 2). Allopatric char had significantly smaller paired cones than sympatric char ($P < 0.05$, Table 2). There was no sig-

TABLE 2. Comparison of cross-sectional cone cell size (±95% CL) at the ellipsoid segment in the retinas of sympatric and allopatric populations of cutthroat trout and Dolly Varden char. L = long axis, S = short axis.

Species	Cone size (μm)		
	Single cone	Paired cones	
		L	S
Sympatric (Loon Lake)			
Trout	5.5±0.1	15.4±0.3	8.8±0.1
Char	7.7±0.1	20.9±0.2	13.2±0.2
Allopatric			
Trout (Placid Lake)	5.4±0.2	15.4±0.3	8.9±0.2
Char (Dickson Lake)	6.3±0.1	17.1±0.2	10.3±0.1

nificant difference ($P > 0.05$) in the length of either axis between sympatric and allopatric trout populations.

The density of photoreceptor cells in the retina of sympatric trout was significantly lower ($P < 0.05$) than that in sympatric char (Table 3). Conversely, the density of bipolar and ganglion cells was significantly higher ($P < 0.05$) in sympatric trout compared with sympatric char. Thus, the summation ratio was lower in sympatric trout (39.6 visual cells for each ganglion cell) compared with sympatric char (78.0:1). There was no significant difference ($P > 0.05$) in the density of any of the cell types between the sympatric and allopatric trout populations, and summation ratios were similar. Estimates of the visual, bipolar, and ganglion cell densities were significantly different ($P < 0.05$) between the sympatric and allopatric char populations as was the degree of summation. The ratio of the number of visual cells to each ganglion cell in the sympatric char population was 78.0:1, while allopatric char had 52.0 visual cells for each ganglion cell.

Qualitatively, there was no difference in the cone cell mosaic among the four populations examined. Tangential sections passing through the cone ellipsoids revealed a pattern of paired cones arranged in squares around a central single cone in all fish examined (Fig. 1C). Although the mosaic was the same, the area occupied by one unit (i.e. four paired cones surrounding a single cone) was not. In particular, there was a significant difference ($P < 0.05$) in the size of one unit between sympatric trout and char and also between sympatric and allopatric char. The differences in the area of one unit of the cone cell mosaic among the four populations were the result of differences in cone cell size described above (Table 2).

Discussion

The results of our study show significant differences in retinal visual cell density between sympatric trout and char and sympatric and allopatric char. Although these differences were based only on an examination of the central portion of the retina and do not offer any insight into variation in visual cell density within a retina as is widely observed in other species (Ahlbert 1976; Ali and Anctil 1976), they are consistent with and may help explain differences in the visual capabilities of the two species (Henderson and Northcote 1985) which have bearing on their distribution and feeding in lakes (Andrusak and Northcote 1971; Hume and Northcote 1985). Further, it is of interest to note that summation ratios of trout and char examined in our study are somewhat higher than those shown for other fish species although the density of visual cells is comparable with that found in other teleosts (e.g. Anctil 1969; Wagner et al. 1976). For example, the summation ratio (i.e. photoreceptor:bipolar:ganglion cells) for walleye (*Stizostedion vitreum vitreum*) ranges from approximately 132:6:1 to 172:6:1 depending on the part of the retina examined (Ali and Anctil 1977). Similar estimates from the same study for the closely related sauger (*Stizostedion canadense*) lie between 126:4:1 and 182:4:1. In contrast, summation ratios in our study ranged from approximately 40:16:1 for sympatric and allopatric trout to 78:22:1 for allopatric char (Table 3). None of the differences noted in our study between retinal structures of trout and char were related to the size or age of fish within the range examined.

Visual acuity under high irradiance conditions, or the distance at which a fish can first detect a target, should be determined in part by the density of cone cells in the retina. This would be expected because image lines can only be resolved when they fall on cones separated by at least one unstimulated cone (Tamura and Wisby 1963). The higher density of cone cells and the smaller cross-sectional size of both paired and single cones in the retina of sympatric trout compared with sympatric char (Table 1) suggest a higher visual acuity in the trout, supporting experimental observations of reactive distance in these sympatric populations (Henderson and Northcote 1985).

Our previous study indicated that the visual system of char would be more effective under low irradiance conditions than would that of trout. In low irradiance environments, fine detail cannot be resolved. One means of enhancing sensitivity to irradiance would be to increase the effective irradiance capture area of each visual receptor. This could be accomplished by enlarging the cross-sectional area of the visual cells. In the family Percidae the cone cells of *Stizostedion vitreum vitreum*, which

TABLE 3. Comparison of the density of nuclei per cell type (±95% CL) and ratios of summation (photoreceptor:bipolar:ganglion cells) in the retinas of sympatric and allopatric populations of cutthroat trout and Dolly Varden char.

Species	Nuclei (1.0 mm^{-2})			
	Photoreceptor	Bipolar	Ganglion	Summation ratio
Sympatric (Loon Lake)				
Trout	125 517±2127	52 542±1751	3169±375	39.6:16.6:1.0
Char	133 440±2043	38 155±1668	1710±334	78.0:22.3:1.0
Allopatric				
Trout (Placid Lake)	126 351±1918	52 959±1960	3461±56	36.5:15.3:1.0
Char (Dickson Lake)	130 104±1626	44 619±1709	2502±292	52.0:17.8:1.0

makes crepuscular and nocturnal feeding forays (Ryder 1977), are much larger than the cones of *Perca flavescens* which is a shallow-water, daytime feeder (Ali et al. 1977). The cross-sectional size of both single and paired cones is greater for sympatric char than sympatric trout (Table 2), suggesting that the visual system of char would be more sensitive under low irradiance conditions.

The effective capture area of a photoreceptor also can be increased by connecting a number of receptors in the same area of the retina together so they function as a unit. The concept of spatial resolution in the retina is usually referred to as summation and has been measured in terms of the number of photoreceptor cells per bipolar cell and the number of bipolar cells per ganglion cell (Anctil 1969). However, because of additional cell types in the retina (e.g. horizontal and amacrine cells), there can be lateral retinal communication so that counts and ratios of receptor, bipolar, and ganglion cells (Table 3) do not completely indicate the degree of summation. Although sensitivity is increased as the level of summation increases, acuity is reduced. In general, the degree of summation is highest for fish living in low irradiance environments (Anctil 1969; Ali and Anctil 1977). In our study, retinas of both sympatric and allopatric forms of trout exhibited a lower level of summation than those of sympatric char. Bearing in mind the above qualifications, our retinal results suggest, as do those of our earlier behavioural study, that sympatric char are more sensitive to low irradiance than either sympatric or allopatric trout.

The preceding discussion has focussed on the probable dependence between several features of the retina measured in our study and visual acuity and sensitivity to irradiance. However, a visual system that is very acute and/or sensitive to irradiance is not necessarily of greatest importance to the fish. For example, the visual system of some fish species may be developed in such a way as to maximize the ability to detect motion or contrast of biologically important surfaces (e.g. prey), independent of acuity and sensitivity. Behavioural studies with *Salmo gairdneri* (Ware 1971) and *Lepomis gibbosus* (Confer and Blades 1975) have shown that these species respond to moving prey targets at a greater distance than to stationary targets of the same size. In *Gadus morhua*, prey movement has been shown to stimulate feeding activity (Brawn 1969). Increasing the contrast of prey targets with respect to the background has also been shown to increase the distance at which they are first detected for *Lepomis gibbosus* (Confer and Blades 1975) and *Salvelinus namaycush* (Kettle and O'Brien 1978). Consequently, it is probable that the visual systems of trout and char are not solely designed to maximize acuity and sensitivity to irradiance but also to make use of other features of biologically important surfaces.

Behavioural studies on visual sensitivity and acuity under different irradiances were only conducted on sympatric populations of trout and char (Henderson and Northcote 1985). However, there is information on the vertical distribution of the allopatric forms. Unlike in sympatry where trout occurred most frequently at the surface and char in deeper waters, allopatric trout and char were commonly found throughout the water column (Andrusak and Northcote 1971). The difference in vertical distribution was most evident between the two forms of char, with allopatric char, unlike the sympatric form, undergoing a diel vertical migration and commonly occurring in the upper 5 m of the water column during daylight. Consequently, one might expect that there would be differences in retinal structure between allopatric and sympatric char, with the former showing characteristics adapted to higher irradiance regimes. Estimates of cone density (Table 1), cone cross-sectional size (Table 2), and degree of summation (Table 3) for allopatric char, intermediate between those obtained for sympatric char and trout, give support to that possibility.

Differences in retinal structure between sympatric trout and char suggest that trout are less sensitive to low irradiance conditions than char but that their visual acuity is better. These observations fit with those of the behavioural experiments (Henderson and Northcote 1985) and may help to explain differences in the vertical distribution and feeding of the two species when in sympatry. Retinal structure of allopatric char is intermediate between that of sympatric trout and char, at least when measured in terms of cone density, cone size, and degree of summation. Again, these estimates correlate with known behavioural differences, allopatric char having a vertical distribution and food habits intermediate between those of sympatric trout and char. There are no apparent differences in retinal structure between sympatric and allopatric trout. Although the vertical distribution of the two forms is not identical, the difference is small, allopatric trout being caught in greatest number between 4 and 5 m and sympatric trout in the upper 3 m of the water column (Andrusak and Northcote 1971). Probably, therefore, the difference in irradiance level experienced by the two forms of trout in the systems examined is not sufficient to cause differences in retinal structure.

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ANNOUNCEMENTS/AVIS

Canadian Conference for Fisheries Research (CCFFR)

Quebec City, Quebec, January 4–5, 1989
Announcement of Conference and Call for Papers

This conference will be held at the Quebec City Aquarium, January 4 and 5, 1989. The major themes of this conference will be (1) measuring the health of fisheries habitats, (2) oceanographic aspects of aquaculture, and (3) environmental control of freshwater and marine production in relation to fisheries.

While papers on other topics may be submitted, authors are encouraged to adhere to these themes as much as possible.

Deadline for abstracts is August 30, 1988. They should be typed (maximum 200 words, single spaced) and the type of presentation (oral/poster) indicated.

Abstracts should be addressed to Dr. Louis Fortier, GIROQ, Department of Biology, Laval University, St. Foy, Que. G1K 7P4.

Conférence canadienne sur la recherche des pêches (CCRP)

Québec (Québec), les 4 et 5 janvier 1989
Avis de conférence et demande de résumés

Cette conférence aura lieu les 4 et 5 janvier 1989 à l'aquarium de Québec. La conférence portera sur les principaux thèmes suivants : 1) la mesure de l'état de santé de l'habitat du poisson; 2) les aspects océanographiques de l'aquiculture; et 3) le contrôle environnemental de la production en eaux douces et marines, en rapport avec les pêches.

On peut présenter des études sur d'autres sujets quoiqu'on encourage les chercheurs à s'en tenir autant que possible à ces thèmes.

La date limite d'envoi de résumés a été fixée au 30 août 1988. Le résumé doit être dactylographié (200 mots au maximum, simple interligne) et l'on doit préciser le genre de présentation (prés. orale/affiche).

Veuillez faire parvenir votre résumé à M. Louis Fortier, D.Ph., GIROQ, Département de biologie, Université de Laval, Ste-Foy (Qué) G1K 7P4.

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